United States Patent [19]
Nelson

[11] Patent Number: 5,256,790
[45] Date of Patent: Oct. 26, 1993

[54] 27-HYDROXYRAPAMYCIN AND DERIVATIVES THEREOF

[75] Inventor: Frances C. Nelson, Yardley, Pa.
[73] Assignee: American Home Products Corporation, New York, N.Y.

[21] Appl. No.: 9,605
[22] Filed: Jan. 27, 1993

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 930,124, Aug. 13, 1992, abandoned.

[51] Int. Cl.[5] C07D 498/16; A61K 31/695
[52] U.S. Cl. 514/291; 540/456
[58] Field of Search 540/456; 514/291

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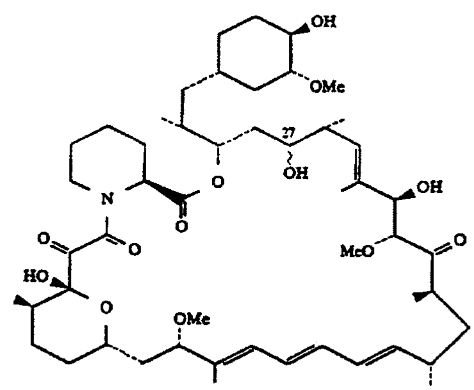
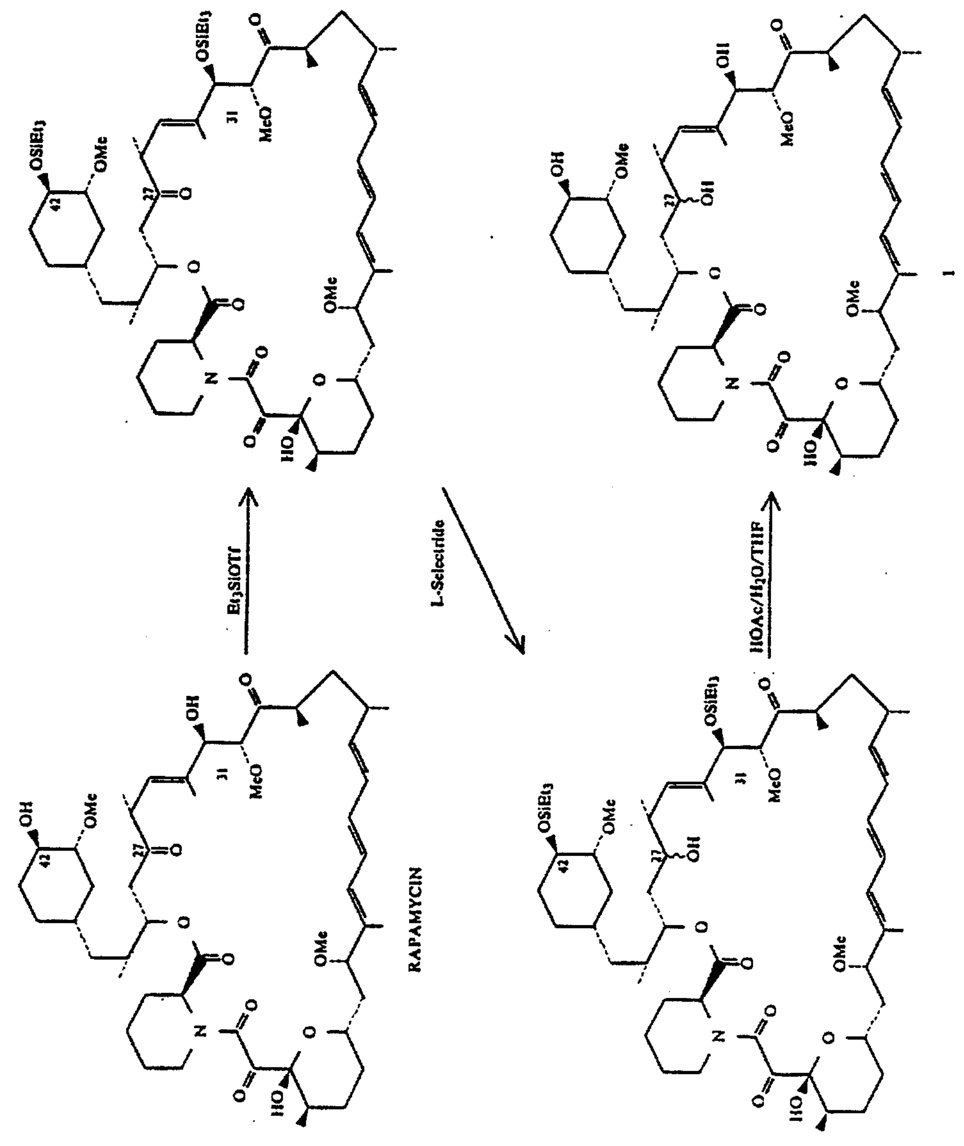
[57] ABSTRACT

This invention provides a compound of formula I,



and 27-substituted derivatives thereof which are useful as immunosuppressive, antiinflammatory, antifungal, antitumor, and antiproliferative agents. The compound depicted by formula I is named 27-hydroxyrapamycin, and may also be referred to as 27-deoxo-27-hydroxyrapamycin.

11 Claims, No Drawings


RAPAMYCIN
Et_3SiOTf
L-Selectride
$HOAc/H_2O/THF$
1

27-Hydroxyrapamycin-27-ester with (2,4-difluorophenyl)carbamic acid

27-Hydroxyrapamycin-27-ester with N-[(1,1-dimethylethoxy)carbonyl]-glycylglycine

27-Hydroxyrapamycin-27-ester with N-[(1,1-dimethylethoxy)carbonyl]-N-methylglycine

27-Hydroxyrapamycin-27-ester with 5-(1,1-dimethylethoxy)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid

27-Hydroxyrapamycin-27-ester with 2-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy)-butanoic acid

27-Hydroxyrapamycin-27-ester with 3-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy)-butanoic acid

27-Hydroxyrapamycin-27-ester with 5-(1,1-dimethyloxy)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid

27-Hydroxyrapamycin-27-ester with N^{α}, N^{ϵ}-bis[(1,1-dimethylethoxy)carbonyl]-L-lysine

27-Hydroxyrapamycin-27-ether with (1-methoxy-1-methyl)ethanol

27-Hydroxyrapamycin-27-ether with (2-(trimethylsilyl)ethoxy)methanol

27-Hydroxyrapamycin-27-ester with N,N-dimethylglycine

27-Hydroxyrapamycin-27-ester with 3-(N,N-diethylamino)propionic acid

27-Hydroxyrapamycin-27-ester with 4'-(N-pyrrolidino)butyric acid

27-Hydroxyrapamycin-27-ester with phenylsulfonylcarbamic acid

27-Hydroxyrapamycin-27-ester with (4-chlorophenylsulfonyl)carbamic acid

27-Hydroxyrapamycin-27-ester with (3-methylphenylsulfonyl)carbamic acid

27-Hydroxyrapamycin-27-ester with 5-(dimethylamino)-1-naphthalensulfonic acid

27-Hydroxyrapamycin-27-ester with 4-methylbenzenesulfonic acid

27-Hydroxyrapamycin-27-ester with 2-thiophenesulfonic acid

27-Hydroxyrapamycin-27-ester with 4-[[4-(dimethylamino)phenyl]aza]benzenesulfonic acid

27-Hydroxyrapamycin-27-ester with 1-naphthalenesulfonic acid

27-Hydroxyrapamycin-27-ester with 8-quinolinsulfonic acid

27-Hydroxyrapamycin-27-ester with methanesulfonic acid

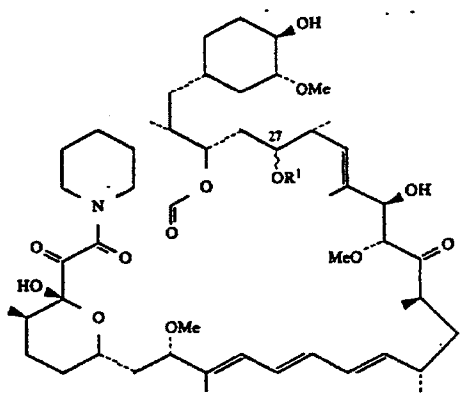
27-Hydroxyrapamycin-27-ester with 2,2,2-trifluoroethanesulfonic acid

27-Hydroxyrapamycin-27-ester with [(methoxycarbonyl)amino]sulfonic acid

What is claimed is:

1. A compound of the formula

II



wherein
R^1 is

$$-\overset{O}{\overset{\|}{C}}R^2$$

and

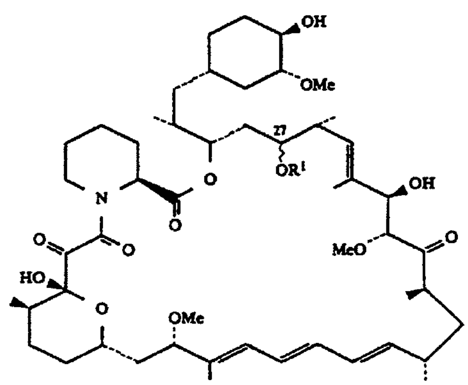
R^2 is alkyl of 1–10 carbon atoms, arylalkyl of 7–10 carbon atoms, or aryl wherein the aryl group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, —SO_3H, —PO_3H, and —CO_2H;

or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 which is 27-hydroxyrapamycin-27-ester with acetic acid.

3. A compound of the formula

III



wherein
R^1 is

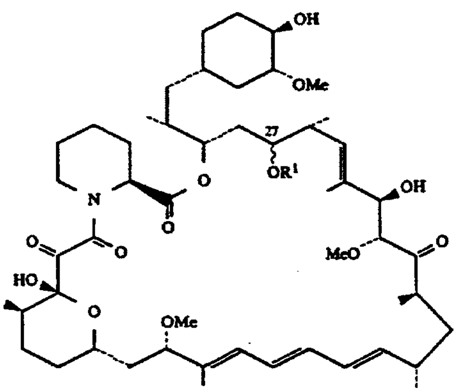
$$-\overset{O}{\overset{\|}{C}}R^2$$

and

R^2 is a mono-, di-, poly-, or per-fluorinated alkyl group of 1-10 carbon atoms.

4. A compound of the formula

IV



wherein

R^1 is

$$-\overset{O}{\overset{\|}{C}}R^2;$$

R^2 is

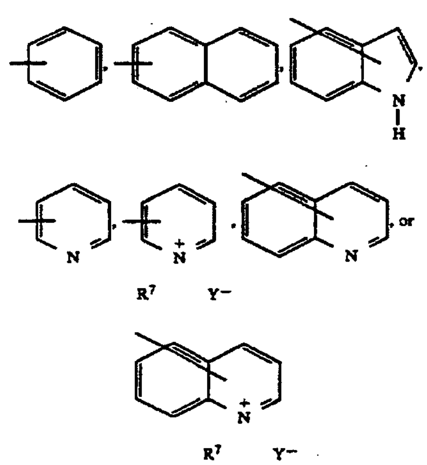
$$-X-\overset{O}{\overset{\|}{C}}-NR^3R^4;$$

X is $-(CH_2)_m-$ or $-Ar-$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1-12 carbon atoms, $-(CH_2)_n-Ar$, $-(CH_2)_p-NR^5R^6$, or $-(CH_2)_p-N^+R^5R^6R^7Y^-$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1-12 carbon atoms, or $-(CH_2)_n-Ar$;

Ar is an optionally mono- or di-substituted group selected from



in which the optional substituents are selected from the group consisting of alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkoxy of 1-6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, or perfluoroalkyl of 1-6 carbon atoms;

R^7 is alkyl of 1-6 carbon atoms;

Y is a halide, sulfate, phosphate, or p-toluenesulfonate anion;

m=1-6;

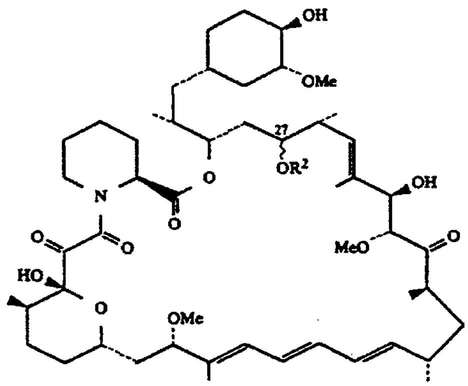
n=1-6;

p=1-6;

or a pharmaceutically acceptable salt thereof.

5. A compound of the formula

VI



wherein

R^2 is

$$-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^3}{\underset{|}{C}}H(CH_2)_n\underset{R^4}{\underset{|}{N}}]_pCO_2R^5;$$

R^3 is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^6$, $-(CH_2)_rNR^7CO_2R^8$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

R^4 and R^7 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms;

R^5, R^6, and R^8 are each, independently, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

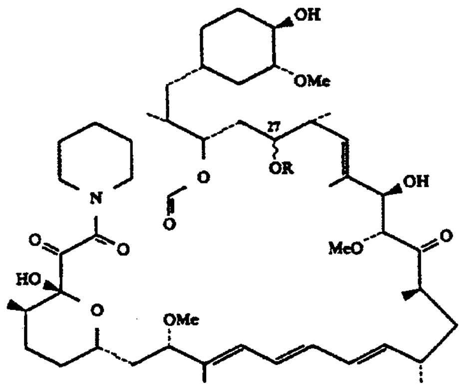
m is 0-4;

n is 0-4;

p is 1-2;

q is 0-4;

r is 0-4;



wherein

R is $-SO_2R^1$;

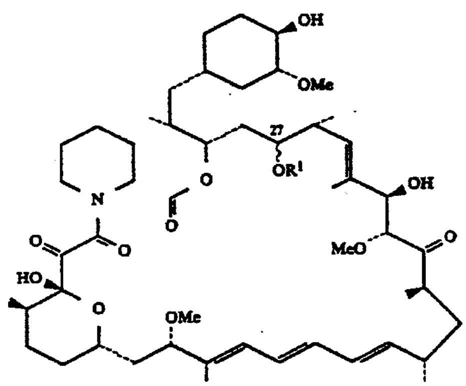
R^1 is alkyl, alkenyl, alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from the group consisting of phenyl and naphthyl or a heterocyclic moiety selected from the group consisting of thiophenyl and quinolinyl; or $-NHCOR^2$; and

R^2 is lower alkyl containing 1 to 6 carbon atoms;

or a pharmaceutically acceptable salt thereof.

10. A method of inducing immunosuppression which comprises administering an immunosuppressive effective amount of a compound of formula II,

II



wherein

R^1 is

$$-\overset{\overset{\large O}{\|}}{C}R^2$$

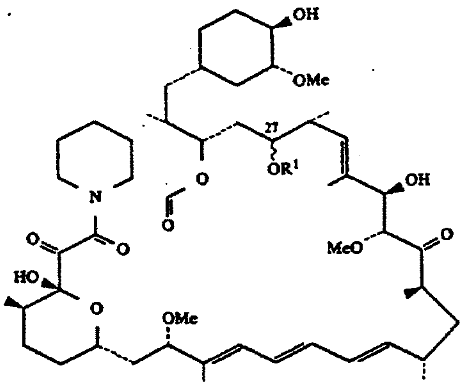
and

R^2 is alkyl of 1–10 carbon atoms, arylalkyl of 7–10 carbon atoms, or aryl wherein the aryl group may be optionally mono-, di-, or tri- substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof;

or of formula III,

III



wherein

R^1 is

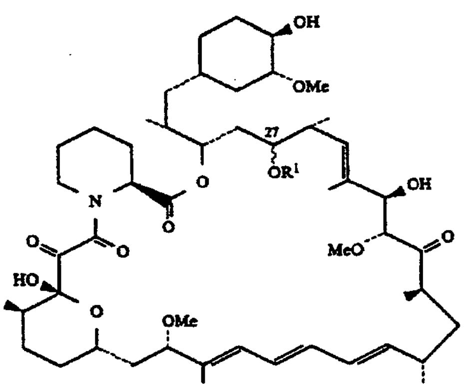
$$-\overset{\overset{\large O}{\|}}{C}R^2$$

and

R^2 is a mono-, di-, poly-, or per-fluorinated alkyl group of 1–10 carbon atoms;

or of formula IV,

IV



wherein

R^1 is

$$-\overset{\overset{\large O}{\|}}{C}R^2;$$

R^2 is

$$-X-\overset{\overset{\large O}{\|}}{C}-NR^3R^4;$$

X is $-(CH_2)_m-$ or $-Ar-$;

R^1 is alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, $—CH_2YX$, $—C(CH_3)_2YX$, $—CH(CH_3)YX$, or L;

Y is O or S;

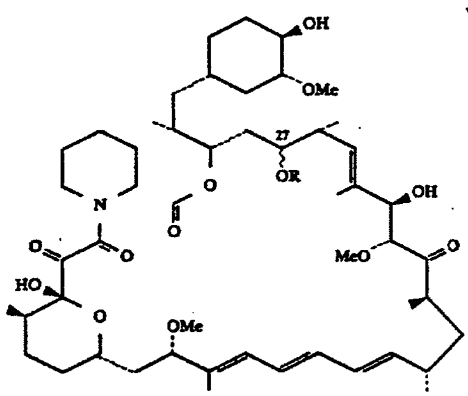
X is $—CH_3$, $—(CH_2)_nCH_3$, $—CH_2Ar$, $—(CH_2)_2OCH_3$, $—CH_2CCl_3$, $—CH(CH_3)_2$, or $—CH_2CH_2SiMe_3$;

L is tetrahydrofuran-2-yl, tetrahydrothiophen-2-yl, tetrahydrothiopyran-2-yl, tetrahydropyran-2-yl, 4-methoxytetrahydropyran-2-yl, 4-methoxytetrahydrothiopyran-2-yl, or 4-methoxytetrahydrothiopyran-2-yl S,S dioxide; and

n=1–5;

or of formula VIII,

VIII



wherein

R is

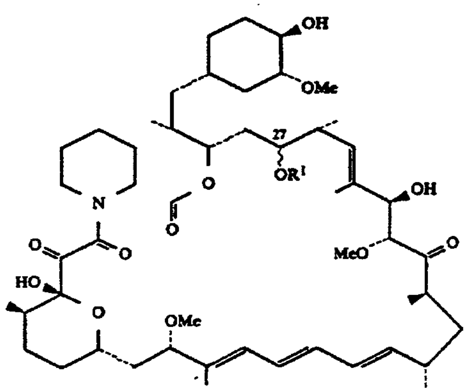
$$—\overset{O}{\overset{\|}{C}}(CH_2)_mNR^1R^2;$$

R^1 and R^2 are each hydrogen or alkyl of 1–3 carbon atoms or R^1 and R^2 together with the nitrogen to which they are attached form a saturated heterocyclic ring having 4–5 carbon atoms; and

m=1–3 or a pharmaceutically acceptable salt thereof;

or of formula IX,

IX



wherein

R^1 is $—CONHSO_2—Ar$; and

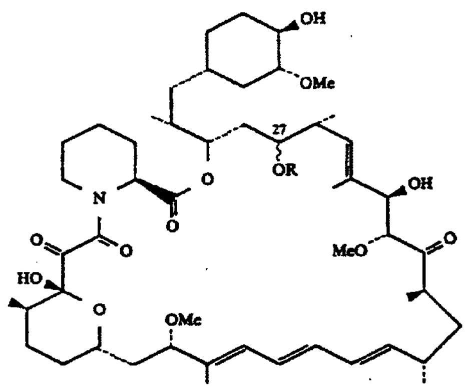
Ar is phenyl, naphthyl, pyridyl, quinolyl, isoquinolyl, quinoxalyl, thienyl, thionaphthyl, furyl, benzofuryl, benzodioxyl, benzoxazolyl, benzoisoxazolyl, or benzodioxolyl; wherein the Ar group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $—SO_3H$, $—PO_3H$, and $—CO_2H$;

or a pharmaceutically acceptable salt thereof when the Ar group contains a basic nitrogen or when the Ar group is substituted by dialkylamino of 1–6 carbon atoms per alkyl group, $—SO_3H$, $—PO_3H$, or $—CO_2H$;

or a pharmaceutically acceptable salt thereof;

or of formula X,

X



wherein

R is $—SO_2R^1$;

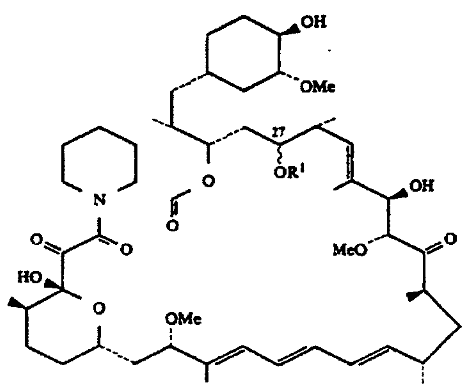
R^1 is alkyl, alkenyl, alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from the group consisting of phenyl and naphthyl or a heterocyclic moiety selected from the group consisting of thiophenyl and quinolinyl; or $—NHCOR^2$; and

R^2 is lower alkyl containing 1 to 6 carbon atoms;

or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition which comprises an effective amount of a compound of formula II,

II



wherein

R^1 is

$$-\overset{O}{\overset{\|}{C}}R^2$$

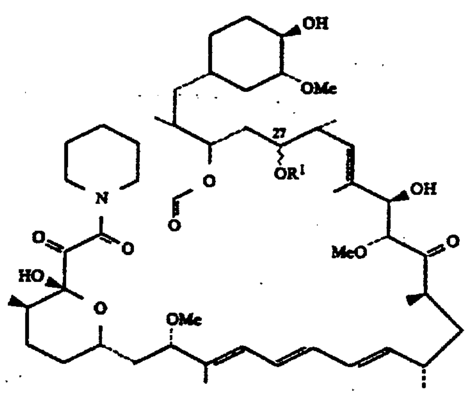
and

R^2 is alkyl of 1–10 carbon atoms, arylalkyl of 7–10 carbon atoms, or aryl wherein the aryl group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof;

or of formula III,

III



wherein

R^1 is

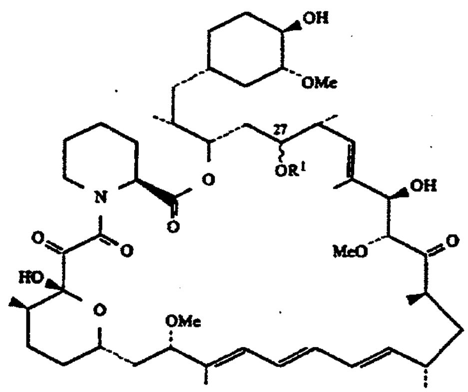
$$-\overset{O}{\overset{\|}{C}}R^2$$

and

R^2 is a mono-, di-, poly-, or per-fluorinated alkyl group of 1–10 carbon atoms;

or of formula IV,

IV



wherein

R^1 is

$$-\overset{O}{\overset{\|}{C}}R^2;$$

R^2 is

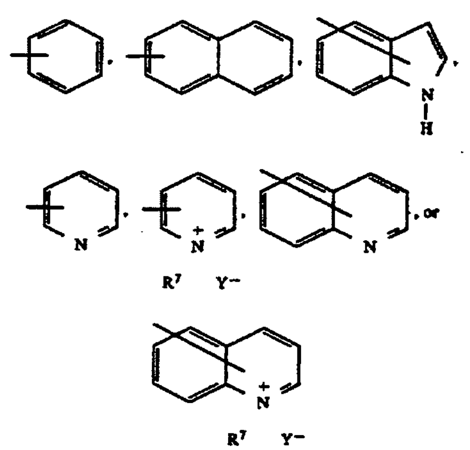
$$-X-\overset{O}{\overset{\|}{C}}-NR^3R^4;$$

X is $-(CH_2)_m-$ or $-Ar-$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, $-(CH_2)_n-Ar$, $-(CH_2)_p-NR^5R^6$, or $-(CH_2)_p-N^+R^5R^6R^7Y^-$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, or $-(CH_2)_n-Ar$;

Ar is an optionally mono- or di-substituted group selected from



in which the optional substituents are selected from the group consisting of alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, or perfluoroalkyl of 1–6 carbon atoms;

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United States Patent [19]

Mitchell et al.

[11] Patent Number: 5,288,711

[45] Date of Patent: Feb. 22, 1994

[54] METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE

[75] Inventors: **Robert D. Mitchell**, Doylestown, Pa.; **Stephen Skwish**, Mercer, N.J.

[73] Assignee: **American Home Products Corporation**, Madison, N.J.

[21] Appl. No.: **874,895**

[22] Filed: **Apr. 28, 1992**

[51] **Int. Cl.**[5] **A61K 31/71**; A61K 31/725

[52] **U.S. Cl.** **514/56**; 514/291; 424/122

[58] **Field of Search** 424/122; 514/291, 56

[56] **References Cited**

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5,078,999	1/1992	Warner	424/122
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Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract) (1990)-Baeder.

Primary Examiner—Douglas W. Robinson
Assistant Examiner—Jean C. Witz
Attorney, Agent, or Firm—Arnold S. Milowsky

[57] **ABSTRACT**

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of a combination of rapamycin and heparin.

13 Claims, No Drawings

METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE

BACKGROUND OF THE INVENTION

Many individuals suffer from heart disease caused by a partial blockage of the blood vessels that supply the heart with nutrients. More severe blockage of blood vessels in such individuals often leads to hypertension, ischemic injury, stroke, or myocardial infarction. Typically vascular occlusion is preceded by vascular stenosis resulting from intimal smooth muscle cell hyperplasia. The underlying cause of the intimal smooth muscle cell hyperplasia is vascular smooth muscle injury and disruption of the integrity of the endothelial barrier and the underlying extracellular matrix. The overall disease process can be termed a hyperproliferative vascular disease because of the etiology of the disease process. Under normal circumstances, the cells of the arterial wall can be looked at as being under stringent negative control and in a quiescent non-proliferating state, probably the consequence of contact with their specialized extracellular matrix. Desquamation of the endothelium, resulting in exposure of and possible disruption of the integrity of the extracellular matrix surrounding the cells, leads to 1) a shift in smooth muscle phenotype from a quiescent, contractile state to a migrating, proliferative form [Manderson, J. A., Arterio 9: (3) (1989)], 2) eventual migration of transformed smooth muscle cells from the medial layer to the sub-lesion intimal layer [Clowes, A. W., Circ. Res. 56: 139 (1985)] and 3) subsequent massive proliferation of the intimal smooth muscle layer resulting in arterial luminal blockage [Clowes, A. W., J. Cardiovas. Pharm. 14 (Suppl 6): S12 (1989)]. Investigations of the pathogenesis of intimal thickening have shown that, following arterial injury, platelets, endothelial cells, macrophages and smooth muscle cells release paracrine and autocrine growth factors (such as platelet derived growth factor, epidermal growth factor, insulin-like growth factor, and transforming growth factor) and other cytokines that result in the smooth muscle cell proliferation and migration. T-cells and macrophages also migrate into the neointima. [Haudenschild, C., Lab. Invest. 41: 407 (1979); Clowes, A., Circ. Res. 56: 139 (1985); Clowes, A., J, Cardiovas. Pharm. 14 (Suppl. 6): S12 (1989); Manderson, J., Arterio. 9: 289 (1989); Forrester, J., J. Am. Coll. Cardiol. 17: 758 (1991)]. This cascade of events is not limited to arterial injury, but also occurs following injury to veins and arterioles.

Vascular injury causing intimal thickening can be broadly categorized as being either biologically or mechanically induced. Atherosclerosis is one of the most commonly occurring forms of biologically mediated vascular injury leading to stenosis. The migration and proliferation of vascular smooth muscle plays a crucial role in the pathogenesis of atherosclerosis. Atherosclerotic lesions include massive accumulation of lipid laden "foam cells" derived from monocyte/macrophage and smooth muscle cells. Formation of "foam cell" regions is associated with a breech of endothelial integrity and basal lamina destruction. Triggered by these events, restenosis is produced by a rapid and selective proliferation of vascular smooth muscle cells with increased new basal lamina (extracellular matrix) formation and results in eventual blocking of arterial pathways. [Davies, P. F., Artherosclerosis Lab. Invest. 55: 5 (1986)].

Until recently, it was generally believed that this proliferation resulted from growth factors released from platelets deposited on the newly exposed matrix surface. However, recent data suggests that this phenomena occurs as a consequence of an intimate interplay between at least three components of the extracellular matrix which act strongly to influence smooth muscle cell phenotype and/or response. These components include: 1) matrix collagen and its subtypes, 2) matrix bound growth factors such as fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β), and 3) the matrix bound proteoglycans, predominantly those containing heparan sulfate glycosaminoglycan chains.

Mechanical injuries leading to intimal thickening result following balloon angioplasty, vascular surgery, transplantation surgery, and other similar invasive processes that disrupt vascular integrity. Intimal thickening following balloon catheter injury has been studied in animals as a model for arterial restenosis that occurs in human patients following balloon angioplasty. Clowes, Ferns, Reidy and others have shown that deendothelialization with an intraarterial catheter that dilates an artery injures the innermost layers of medial smooth muscle and may even kill some of the innermost cells. [Schwartz, S. M., Human Pathology 18: 240 (1987); Fingerle, J., Arteriosclerosis 10: 1082 (1990)] Injury is followed by a proliferation of the medial smooth muscle cells, after which many of them migrate into the intima through fenestrae in the internal elastic lamina and proliferate to form a neointimal lesion.

Vascular stenosis can be detected and evaluated using angiographic or sonographic imaging techniques [Evans, R. G., JAMA 265: 2382 (1991)] and is often treated by percutaneous transluminal coronary angioplasty (balloon catheterization). Within a few months following angioplasty, however, the blood flow is reduced in approximately 30–40 percent of these patients as a result of restenosis caused by a response to mechanical vascular injury suffered during the angioplasty procedure, as described above. [Pepine, C., Circulation 81: 1753 (1990); Hardoff, R., J. Am. Coll. Cardiol. 15 1486 (1990)].

It has been shown that heparin inhibits smooth muscle cell growth both in culture and in vivo. [Tiozzo, R., Arzneim. Forsch./Drug. Res. 39: 15 (1989)]; [Clowes, A. W., Circ. Res. 58 (6): 839 (1986); Clowes, A. W., Circ. Res. 56: 139 (1985)]. As early as 1977, Clowes and Karnovsky [Clowes, A. W., Nature 265: 625 (1977)] showed that administration of commercial heparin to animals whose carotid arteries have been injured in order to produce a myointimal plaque dramatically reduced the size of the myointimal thickening. The authors, showed that the effect of heparin on the injured arterial wall was to inhibit the growth of smooth muscle cells and that this effect was, in no way, related to the anti-coagulant activity of the heparin. Heparin, through its obligatory role in promoting growth factor binding, also has been shown to promote endothelial growth, a necessary element of vascular healing following vascular injury. [Bjornsson, M., Proc. Natl. Acad. Sci. USA 88: 8651 (1991); Klagsburn, M., Cell 67: 229 (1991); Klein-Soyer, C., Arterio. 9: 147 (1989); Lindner, V. J. Clin. Invest. 85: 2004 (1990); Ornitz, D. M., Molecular and Cellular Biology 12: 240 (1992); Saksela, O., J. Cell Biol. 107: 743 (1988); Thornton, S. C., Science 222: 623 (1983)]. De Vries has also reported efficacious results in preventing restenosis in clinical studies with heparin

[Eur. Heart J. 12 (Suppl.): 386 (1991)], however, Lehmann reported that chronic use of heparin (1000 units/-day, s.c.) after successful coronary angioplasty paradoxically appears to increase the likelihood of restenosis, and caused abnormal bleeding in 41% of patients in the study. [JACC 17(2): 181A (1991)].

Rapamycin, a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus* [U.S. Pat. No. 3,929,992] has been shown to prevent the formation of humoral (IgE-like) antibodies in response to an albumin allergic challenge [Martel, R., Can. J. Physiol. Pharm. 55: 48 (1977)], inhibit murine T-cell activation [Staruch, M., FASEB 3: 3411 (1989)], prolong survival time of organ grafts in histoincompatible rodents [Morris, R., Med. Sci. Res. 17: 877 (1989)], and inhibit transplantation rejection in mammals [U.S. Pat. No. 5,100,899]. Rapamycin has also has been shown to inhibit proliferation of vascular smooth muscle cells in vitro in response to mitogenic and heterotrophic factors, and in vivo following balloon catheterization of the carotid artery. [Morris, R., J. Heart Lung Transplant. 11 (pt. 2): 1992)].

DESCRIPTION OF THE INVENTION

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal in need thereof by administering an antiproliferative effective amount of a combination of rapamycin and heparin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with a combination of rapamycin and heparin.

As such, the combination of rapamycin and heparin is useful in preventing or treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to autoimmune disorders; alloimmune related disorders; infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium.

Preventing includes the prophylactic prevention of hyperproliferative vascular disease in a susceptible mammal and treating includes arresting the development, and retarding the progression of hyperproliferative vascular disease in a susceptible mammal.

Administration can also be accomplished via mixed routes of administration. For example, rapamycin may be given orally and heparin given parenterally. A vascular stent can be impregnated with either rapamycin or heparin, and the other component of the combination can be administered orally or parenterally. Other permutations of mixed modes of administration will be appreciated by one skilled in the art.

The effect of the combination of rapamycin and heparin on hyperproliferative vascular disease was established in a standard pharmacological test procedure that emulates the hyperproliferative effects observed in mammals that are undergoing intimal smooth muscle proliferation and are therefore developing restenosis. The procedure used and the results obtained are described below.

Primary rat aorta smooth muscle cell cultures from passage 2–10 were grown to confluence in 100 mm culture dishes (Falcon, 1029) in media 199 (M199; Gibco 320 1150AJ) plus 10% fetal bovine serum (FBS, Gibco 240 6000AG). Cells were washed with calcium, magnesium free Delbecco's phosphate buffered saline (-D-PBS; Gibco, 310-4190AJ) and trypsinized (Gibco, 610-5050AG) for five minutes. Cells were scraped from culture dishes with a rubber policeman and centrifuged out of enzyme (10 minutes × 1000 g). Cells were resuspended in M199 plus 10% FBS containing (^{3}H)-thymidine (0.5 μCi/mL) at 8–15,000 cells/mL, and were plated into either 24 (Falcon, 3047) or 96 (Costar 9102) well plates (1 mL in 24 well plate and 200 μL in 96 well plates.) Drugs were added to each well (20 μL in 24 well plates and 4 μL in 96 well plate; 50 fold dilution) and plates were incubated for 24 hours at 37°; 5% CO_2. Plates were placed on ice and washed three times with ice cold DeBelco's phosphate buffered saline (D-PBS; Gibco 310-4040AJ) and were incubated in ice cold 10% trichloroacetic acid (TCA) for 30 minutes to remove acid soluble proteins (leaving only cell superstructure and DNA). Plates were washed three times with TCA and aspired dry. 96-well plates were snapped apart and placed in scintillation vials, scintillated (10 mL/vial) and counted. 24-well plates were treated with 0.4N NaOH (250 μL/well) for 3–4 hours to solubilize cells. Solution was transferred to scintillation vials containing 0.4N HCl (250 μL/vial; to neutralize NaOH) and each well was rinsed two times with water (250 μL) for a total volume of 1 mL/vial. Vials were scintillated (10 mL/vial) and counted.

The following table shows the results obtained for the combination of rapamycin and heparin on rat aortic smooth muscle cell proliferation.

EFFECT OF HEPARIN AND RAPAMYCIN ON CULTURED RAT AORTIC SMOOTH MUSCLE CELL PROLIFERATION (VALUES EXPRESSED AS % OF CONTROL ± STANDARD DEVIATION)

RAP	HEPARIN (μg/ml)						
(nM)	0	0.1	1.0	10	25	50	200
0	100 ± 1.3	101.7 ± 5.1	67.2 ± 6.5	53.2 ± 2.2	38.1 ± 1.9	31.5 ± 0.5	25.1 ± 1.9
0.01	100.6 ± 3.1	102.4 ± 4.4	65.1 ± 2.8	47.5 ± 2.0	36.5 ± 0.8	29.1 ± 1.4	26.0 ± 1.7
0.1	97.8 ± 2.7	104.6 ± 7.0	61.0 ± 5.2	43.9 ± 3.3	29.7 ± 2.5	23.8 ± 0.7	20.1 ± 1.0
1.0	70.1 ± 1.7	75.7 ± 4.3	45.6 ± 5.9	24.4 ± 0.4	13.7 ± 0.3	12.0 ± 0.3	9.7 ± 0.6
10.0	56.0 ± 2.9	53.7 ± 2.3	28.7 ± 2.4	17.9 ± 0.7	11.1 ± 0.5	4.4 ± 1.4	3.8 ± 1.1
100.0	50.5 ± 3.0	50.0 ± 2.3	29.0 ± 2.2	17.2 ± 1.1	10.1 ± 0.2	4.4 ± 1.2	3.8 ± 0.8

The results of this standard test procedure demonstrates that the combination of rapamycin and heparin

prevented vascular smooth muscle cell proliferation, and is therefore useful in preventing or treating hyperproliferative vascular disease. Specifically, the combination of rapamycin and heparin is useful in preventing or treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury.

While the results also show that rapamycin and heparin each are separately effective in preventing vascular smooth muscle cell proliferation, the combination of rapamycin with heparin is distinctly advantageous over either monotherapy as the combination takes advantage of the beneficial aspects of each agent, while minimizing the negative aspects of each agent. Rapamycin is a relatively nonselective, potent antiproliferative agent which inhibits both intimal smooth muscle cell proliferation as well as endothelial cell growth. As endothelial regrowth is necessary to prevent the occurrence of restenosis following the cessation of treatment, it can be expected the nonselective antiproliferative properties of rapamycin would require lengthy treatment periods to provide for endothelial healing. In contrast, heparin has relatively selective antiproliferative properties. Heparin has been shown to prevent smooth muscle cell growth, while promoting endothelial cell growth, thereby inhibiting intimal narrowing, and promoting vascular endothelial healing. [Bjornsson, M., Proc. Natl. Acad. Sci. USA 88: 8651 (1991); Klagsburn, M., Cell 67: 229 (1991); Klein-Soyer, C., Arterio. 9: 147 (1989); Lindner, V. J. Clin. Invest. 85: 2004 (1990); Ornitz, D. M., Molecular and Cellular Biology 12: 240 (1992); Saksela, O., J. Cell Biol. 107: 743 (1988); Thornton, S. C., Science 222: 623 (1983)]. It has been shown that upon reestablishment of the endothelial layer following vascular injury, intimal smooth muscle cell proliferation ceases and restenosis is therefore arrested. [Reidy, M., Lab. invest. 59: 36 (1988); Chevru, A., Surg. Gynecol. Obstet. 171: 443 (1990); Fishman, J., Lab. Invest. 32: 339 (1975); Haudenschild, C., Lab. Invest 41: 407 (1979)]. Heparin therapy therefore provides the beneficial therapeutic profile of promoting endothelial healing while suppressing intimal smooth muscle cell proliferation. Treatment with heparin, however, is not without side effects. In addition to acting as an antiproliferative agent, heparin is also a powerful anticoagulant, and can cause hemorrhage. [Ellis, S., Am. Heart J. 117: 777 (1989)]. Antibodies to heparin also develop during chronic heparin administration which bind to platelets leading to thrombocytopenia.

As shown in Table 1, the use of rapamycin in combination with heparin provides for dramatically reduced dosages of each agent to produce the same effect. For example, at a combination dose of 1.0 nM rapamycin and 10 μg/mL heparin, smooth muscle cell proliferation occurs is inhibited by 76% (24% of control level), whereas a dose of 200 μg/mL of heparin alone is needed to achieve this degree of inhibition. A dose of 100 nM rapamycin was not able to prevent smooth muscle cell proliferation to this extent. By achieving efficacious results at lower doses of each agent, the negative aspects of each agent can be alleviated. The combined use of rapamycin and heparin allows a minimization of the dose of rapamycin used, as such, the antiproliferative effect on endothelial cell growth is expected to be negated by the proliferative effect of heparin on the endothelium. Additionally, by using lower doses of heparin, the dose dependent side effects associated with heparin can be avoided.

Based on this disclosure, other advantages of using rapamycin in combination with heparin for preventing or treating hyperproliferative vascular disorders will be apparent to one skilled in the art.

When rapamycin is employed in combination with heparin in the prevention or treatment of hyperproliferative vascular disease, it can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Rapamycin in combination with heparin may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. Rapamycin in combination with heparin may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active

compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Rapamycin in combination with heparin can be administered intravascularly or via a vascular stent impregnated with rapamycin in combination with heparin, during balloon catheterization to provide localized effects immediately following injury.

Rapamycin in combination with heparin may be administered topically as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1-5 percent, preferably 2%, of active compound.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily dosages of rapamycin, when administered in combination with heparin, would be 0.005-50 mg/kg and preferably between 0.05-10 mg/kg. Since non-anticoagulant heparin and anticoagulant heparins are equally effective, dosage for heparin should be established on a mg/kg basis preferably between 1-100 mg/kg, noting that at higher combinations of heparin, a non-anticoagulant form is preferred to avoid hemorrhagic side effects.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, intravascular, intranasal, intrabronchial, transdermal, or rectal administration will be determined by the administering physician based on experience with the individual subject treated. In general, the combination of rapamycin and heparin is most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects, and can be administered either as a single unit dose, or if desired, the dosage may be divided into convenient subunits administered at suitable times throughout the day.

What is claimed is:

1. A method of treating vascular disease resulting from smooth muscle cell proliferation in a mammal in need thereof which comprises, administering an antiproliferative effective amount of the combination of rapamycin in a concentration of at least 1 nM and heparin in a concentration of at least 1 μg/ml to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via an impregnated vascular stent.

2. The method according to claim 1 wherein the vascular disease resulting from smooth muscle cell proliferation is intimal smooth muscle cell hyperlasia or restenosis.

3. The method according to claim 2 wherein the combination of rapamycin and heparin is administered concurrent with said mammal undergoing a percutaneous transluminal coronary angioplasty procedure.

4. The method according to claim 3 wherein the hyperproliferative vascular disease is restenosis.

5. The method according to claim 2 wherein the combination of rapamycin and heparin is administered subsequent to said mammal undergoing a percutaneous transluminal coronary angioplasty procedure.

6. The method according to claim 5 wherein the hyperproliferative vascular disease is restenosis.

7. The method according to claim 2 wherein the combination of rapamycin and heparin is administered concurrent with said mammal sustaining a biologically or mechanically mediated vascular injury.

8. The method according to claim 2 wherein the combination of rapamycin and heparin is administered subsequent to said mammal sustaining a biologically or mechanically mediated vascular injury.

9. A composition for the use in preventing or treating hyperproliferative vascular disease in a mammal which comprises an antiproliferative effective amount of a combination of rapamycin in a concentration of at least 1 nM and heparin in a concentration of at least 1 μg/ml and a pharmaceutically acceptable carrier.

10. The composition according to claim 9 wherein the vascular disease resulting from smooth muscle cell proliferation is intimal smooth muscle cell hyperplasia or restenosis.

11. The method according to claim 1 wherein the vascular disease resulting from smooth muscle cell proliferation is vascular occlusion.

12. The composition according to claim 9 wherein the vascular disease resulting from smooth muscle cell proliferation is vascular occlusion.

13. A method of treating restenosis in a mammal resulting from said mammal undergoing a percutaneous transluminal coronary angioplasty procedure which comprises administering an antirestenosis effective amount of a combination of rapamycin and heparin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via an impregnated vascular stent.

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US005362718A

United States Patent [19]

Skotnicki et al.

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[45] Date of Patent: Nov. 8, 1994

[54] RAPAMYCIN HYDROXYESTERS

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[51] Int. Cl.^{5} A61K 31/695; A61K 31/395; C07D 498/16; C07D 7/04

[52] U.S. Cl. 514/63; 514/291; 540/452; 540/456

[58] Field of Search 540/456, 452; 514/291, 514/63

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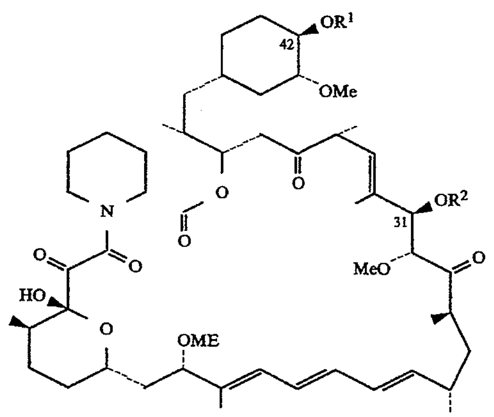
[57] ABSTRACT

A compound of the structure

(Abstract continued on next page.)

A1064



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl, alkenyl, alkynyl, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl, alkenyl, alkynyl, tri-(alkyl)silyl, tri-(alkyl)silylethyl, triphenylmethyl, benzyl, alkoxymethyl, tri-(alkyl)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl, alkenyl, alkynyl, or phenylalkyl;

X is 5-(2,2-dialkyl)[1,3]dioxanyl, 5-(2,2-dicycloalkyl)[1,3]dioxanyl, 4-(2,2-dialkyl)[1,3]dioxanyl, 4-(2,2-dicycloalkyl)[1,3]dioxanyl, 4-(2,2dialkyl)[1,3-]dioxalanyl, or 4-(2,2-dicycloalkyl)[1,3]dioxalanyl;

b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl group, or a pharmaceutically acceptable salt thereof which is useful as an immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agent.

24 Claims, No Drawings

RAPAMYCIN HYDROXYESTERS

BACKGROUND OF THE INVENTION

This invention relates to hydroxyesters of rapamycin and a method for using them for inducing immunosuppression, and in the treatment of transplantation rejection, graft vs. host disease, autoimmune diseases, diseases of inflammation, adult T-cell leukemia/lymphoma, solid tumors, fungal infections, and hyperproliferative vascular disorders.

Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in *vitro* and *in vivo* [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31,539 (1978); U.S. Pat. Nos. 3,929,992; and 3,993,749].

Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

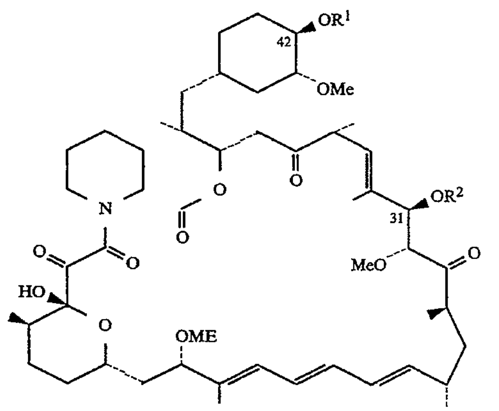
The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Pat. No. 5,100,899].

Rapamycin has also been shown to be useful in preventing or treating systemic lupus erythematosus [U.S. Pat. No. 5,078,999], pulmonary inflammation [U.S. Pat. No. 5,080,899], insulin dependent diabetes mellitus [Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract), (1990)], smooth muscle cell proliferation and intimal thickening following vascular injury [Morris, R. J. Heart Lung Transplant 11 (pt. 2): 197 (1992)], adult T-cell leukemia/lymphoma [European Patent Application 525,960 Al], and ocular inflammation [European Patent Application 532,862 Al].

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble aminoacyl prodrugs of rapamycin (U.S. Pat. No. 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions.

DESCRIPTION OF THE INVENTION

This invention provides derivatives of rapamycin which are useful as immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agents having the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

b=0–6;

d=0–6; and

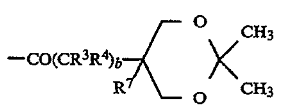
f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

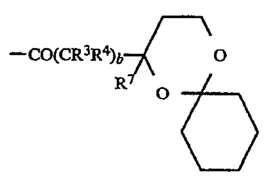
The pharmaceutically acceptable salts are those derived from such inorganic cations such as sodium, potassium, and the like; and organic bases such as: mono-, di-, and trialkyl amines of 1–6 carbon atoms, per alkyl group and mono-. di-, and trihydroxyalkyl amines of 1–6 carbon atoms per alkyl group, and the like.

The terms alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, and alkynyl of 2–7 carbon atoms, include both straight chain as well as branched carbon chains. As the compounds of this invention can contain more than one —$(CR^3R^4)_fOR^{10}$ group, R^3, R^4, f, and R^{10} can be the same or different. Similarly, when other generic substituent descriptions are repeated in the same structure, they can be the same or different.

For a compound in which R^1 contains R^8 and R^9 taken together to form X, where X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, the alkyl group of X contains 1 carbon atom, and d=0, R^1 would have the following structure.



Similarly, for a compound in which R^1 contains R^8 and R^9 taken together to form X, where X is 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, the cycloalkyl group of X contains 6 carbon atom, and d=0, R^1 would have the following structure.



For compounds containing X, preferred compounds include those in which the alkyl group of X, if present, is methyl and the cycloalkyl group of X, if present, is cyclohexyl.

When R^{10} is not hydrogen, alkyl, alkenyl, or alkynyl, it is intended that R^{10} is a group that can serve as an alcohol protecting group. Thus, these groups are intermediates of free hydroxylated compounds, as well as being biologically active in their own right. R^{10} covers tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, and tetrahydropyranyl groups. Other alcohol protecting groups are known by one skilled in the an and are also considered pan of this invention.

Of the compounds of this invention preferred members are those in which R^2 is hydrogen; those in which R^2 is hydrogen, b=0, and d=0; those in which R^2 is hydrogen, b=0, d=0, and R^8 and R^9 are each, independently hydrogen, alkyl, or —$(CR^3R^4)_fOR^{10}$, or are taken together to form X.

Compounds of this invention having the ester group —$CO(CR^3R^4)_bCR^5R^6)_d(CR^7R^8R^9)_e$ at the 42- or 31,42-positions can be prepared by acylation of rapamycin using protected hydroxy and polyhydroxy acids, alkoxy or polyalkoxy carboxylic acids that have been activated, followed by removal of the alcohol protecting groups, if so desired. Several procedures for carboxylate activation are known in the art, but the preferred methods utilize carbodiimides, mixed anhydrides, or acid chlorides. For example, an appropriately substituted carboxylic acid can be activated as a mixed anhydride, with an acylating group such as 2,4,6-trichlorobenzoyl chloride. Treatment of rapamycin with the mixed anhydride under mildly basic condition provides the desired compounds. Alternatively, the acylation reaction can be accomplished with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and dimethylaminopyridine. Mixtures of 42- and 31,42-esters can be separated by chromatography.

The 31-ester-42-hydroxy compounds of this invention can be prepared by protecting the 42-alcohol of rapamycin with a protecting group, such as with a tert-butyl dimethylsilyl group, followed by esterification of the 31-position by the procedures described above. The preparation of rapamycin 42-silyl ethers is described in U.S. Pat. No. B1 5,120,842, which is hereby incorporated by reference. Removal of the protecting group provides the 31-esterified compounds. In the case of the tert-butyl dimethylsilyl protecting group, deprotection can be accomplished under mildly acidic conditions, such as acetic acid/water/THF. The deprotection procedure is described in Example 15 of U.S. Pat. No. 5,118,678, which is hereby incorporated by reference.

Having the 31-position esterified and the 42-position deprotected, the 42-position can be esterified using a different acylating agent than was reacted with the 31-alcohol, to give compounds having different esters at the 31- and 42- positions. Alternatively, the 42-esterified compounds, prepared as described above, can be reacted with a different acylating agent to provide compounds having different esters at the 31-and 42-positions.

This invention also covers analogous hydroxy esters of other rapamycins such as, but not limited to, 29-demethoxyrapamycin, [U.S. Pat. No. 4,375,464, 32-demethoxyrapamycin under C.A. nomenclature]; rapamycin derivatives in which the double bonds in the 1-, 3-, and/or 5-positions have been reduced [U.S. Pat. No. 5,023,262]; 29-desmethylrapamycin [U.S. Pat. No. 5,093,339, 32-desmethylrapamycin under C.A. nomenclature]; 7,29-bisdesmethylrapamycin [U.S. Pat. No. 5,093,338, 7,32-desmethylrapamycin under C.A. nomenclature]; and 15-hydroxyrapamycin [U.S. Pat. No. 5,102,876]. The disclosures in the above cited U.S. Patents are hereby incorporated by reference.

Immunosuppressive activity for representative compounds of this invention was evaluated in an in vitro standard pharmacological test procedure to measure the inhibition of lymphocyte proliferation (LAF) and in two in vivo standard pharmacological test procedures. The pinch skin graft test procedure measures the immunosuppressive activity of the compound tested as well as the ability of the compound tested to inhibit or treat transplant rejection. The adjuvant arthritis standard pharmacological test procedure, which measures the ability of the compound tested to inhibit immune mediated inflammation. The adjuvant arthritis test procedure is a standard pharmacological test procedure for rheumatoid arthritis. The procedures for these standard pharmacological test procedures are provided below.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative com-

pounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated radioactivity is determined. Inhibition of lymphoproliferation is assessed as percent change in counts per minute from nondrug treated controls. For each compound evaluated, rapamycin was also evaluated for the purpose of comparison. An IC_{50} was obtained for each test compound as well as for rapamycin. When evaluated as a comparator for the representative compounds of this invention, rapamycin had an IC_{50} ranging from 0.6–1.5 nM. The results obtained are provided as an IC_{50} and as the percent inhibition of T-cell proliferation at 0.1 μM. The results obtained for the representative compounds of this invention were also expressed as a ratio compared with rapamycin. A positive ratio indicates immunosuppressive activity. A ratio of greater than 1 indicates that the test compound inhibited thymocyte proliferation to a greater extent than rapamycin. Calculation of the ratio is shown below.

$$\frac{IC_{50} \text{ of Rapamycin}}{IC_{50} \text{ of Test Compound}}$$

Representative compounds of this invention were also evaluated in an in vivo test procedure designed to determine the survival time of pinch skin graft from male BALB/c donors transplanted to male C_3H(H-2K) recipients. The method is adapted from Billingham R. E. and Medawar P. B., J. Exp. Biol. 28:385–402, (1951). Briefly, a pinch skin graft from the donor was grafted on the dorsum of the recipient as a allograft, and an isograft was used as control in the same region. The recipients were treated with either varying concentrations of test compounds intraperitoneally or orally. Rapamycin was used as a test control. Untreated recipients serve as rejection control. The graft was monitored daily and observations were recorded until the graft became dry and formed a blackened scab. This was considered as the rejection day. The mean graft survival time (number of days±S.D.) of the drug treatment group was compared with the control group. The following table shows the results that were obtained. Results are expressed as the mean survival time in days. Untreated (control) pinch skin grafts are usually rejected within 6–7 days. Compounds were tested using a dose of 4 mg/kg.

The adjuvant arthritis standard pharmacological test procedure measures the ability of test compounds to prevent immune mediated inflammation and inhibit or treat rheumatoid arthritis. The following briefly describes the test procedure used. A group of rats (male inbread Wistar Lewis rats) are pre-treated with the compound to be tested (1 h prior to antigen) and then injected with Freud's Complete Adjuvant (FCA) in the right hind paw to induce arthritis. The rats are then orally dosed on a Monday, Wednesday, Friday schedule from day 0–14 for a total of 7 doses. Both hind paws are measured on days 16, 23, and 30. The difference in paw volume (mL) from day 16 to day 0 is determined and a percent change from control is obtained. The left hind paw (uninjected paw) inflammation is caused by T-cell mediated inflammation and is recorded in the above table (% change from control). The right hind paw inflammation, on the other hand, is caused by non-

specific inflammation. Compounds were tested at a dose of 5 mg/kg. The results are expressed as the percent change in the uninjected paw at day 16 versus control; the more negative the percent change, the more potent the compound. Rapamycin provided between −70% and −90% change versus control, indicating that rapamycin treated rats had between 70–90% less immune induced inflammation than control rats.

The results obtained in these standard pharmacological test procedures are provided following the procedure for making the specific compounds that were tested.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this invention. The results obtained in the LAF test procedure indicates suppression of T-cell proliferation, thereby demonstrating the immunosuppressive activity of the compounds of this invention. Further demonstration of the utility of the compounds of this invention as immunosuppressive agents was shown by the results obtained in the skin graft and adjuvant arthritis standard pharmacological test procedures. Additionally, the results obtained in the skin graft test procedure further demonstrates the ability of the compounds of this invention to treat or inhibit transplantation rejection. The results obtained in the adjuvant arthritis standard pharmacological test procedure further demonstrate the ability of the compounds of this invention to treat or inhibit rheumatoid arthritis.

Based on the results of these standard pharmacological test procedures, the compounds are useful in the treatment or inhibition of transplantation rejection such as kidney, heart, liver, lung, bone marrow, pancreas (islet cells), cornea, small bowel, and skin allografts, and heart valve xenografts; in the treatment or inhibition of autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; and diseases of inflammation such as psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease, pulmonary inflammation (including asthma, chronic obstructive pulmonary disease, emphysema, acute respiratory distress syndrome, bronchitis, and the like), and eye uveitis.

Because of the activity profile obtained, the compounds of this invention also are considered to have antitumor, antifungal activities, and antiproliferative activities. The compounds of this invention therefore also useful in treating solid tumors, adult T-cell leukemia/lymphoma, fungal infections, and hyperproliferative vascular diseases such as restenosis and atherosclerosis. When used for restenosis, it is preferred that the compounds of this invention are used to treat restenosis that occurs following an angioplasty procedure. When used for this purpose, the compounds of this invention can be administered prior to the procedure, during the procedure, subsequent to the procedure, or any combination of the above.

When administered for the treatment or inhibition of the above disease states, the compounds of this invention can be administered to a mammal orally, parenterally, intranasally, intrabronchially, transdermally, topically, intravaginally, or rectally.

It is contemplated that when the compounds of this invention are used as an immunosuppressive or antiinflammatory agent, they can be administered in conjunction with one or more other immunoregulatory agents.

Such other immunoregulatory agents include, but are not limited to azathioprine, corticosteroids, such as prednisone and methylprednisolone, cyclophosphamide, rapamycin, cyclosporin A, FK-506, OKT-3, and ATG. By combining the compounds of this invention with such other drugs or agents for inducing immunosuppression or treating inflammatory conditions, the lesser amounts of each of the agents are required to achieve the desired effect. The basis for such combination therapy was established by Stepkowski whose results showed that the use of a combination of rapamycin and cyclosporin A at subtherapeutic doses significantly prolonged heart allograft survival time. [Transplantation Proc. 23: 507 (1991)].

The compounds of this invention can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid. When formulated orally, it has been found that 0.01% Tween 80 in PHOSAL PG-50 (phospholipid concentrate with 1,2-propylene glycol, A. Nattermann & Cie. GmbH) provides an acceptable oral formulation.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions ,and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carders are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

The compounds of this invention may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. The compounds of this invention may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound which may be administered to a fungally affected area.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily dosages of active compound would be 0.1 μg/kg–100 mg/kg, preferably between 0.001–25 mg/kg, and more preferably between 0.01–5 mg/kg. Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, nasal, or intrabronchial administration will be determined by the administering physician based on experience with the individual subject treated. Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example., packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form.

The following examples illustrate the preparation and biological activities of representative compounds of this invention.

EXAMPLE 1

Rapamycin 42-ester with (tetrahydropyran-2-yloxy)acetic acid

2,4,6-Trichlorobenzoyl chloride (0.55 mL, 3.51 mmol) was added via syringe to a solution of the glycolic acid THP-ether (0.562 g, 3.51 mmol) and triethylamine (0.49 mL, 3.51 mmol) in 10 mL THF at 0 ° C. under nitrogen. The mixture was stirred for 4 h at room

temperature, and a white precipitate formed. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 10 mL benzene, then rapamycin (2.92 g, 3.19 mmol) and DMAP (0.429 g, 3.51 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with cold 1N HCl (aq), saturated $NaHCO_3$ (aq) and brine, dried over $MgSO_4$, filtered and concentrated to an oily yellow solid. Flash chromatography (2X with 65% EtOAc-hexane) afforded the title compound (1.114 g, 33%) as a white solid.

(−)FAB-MS m/z 1055.5 (M−), 590.3 (southern fragment), 463.2 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.60 (m, 1 H, C(42)H), 4.66 (m, 1H), 4.14 (s, 2H), 3.73 (m, 1H), 3.42 (m, 1H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 169.2, 97.4, 63.5, 61.2, 29.7, 24.8. 18.8.

EXAMPLE 2

Rapamycin 42-ester with hydroxyacetic acid

p-Toluenesulfonic acid (10 mg) was added to a solution of the product of Example 1 (306 mg, 0.29 mmol) in 10 mL CH_3OH at 0 ° C. The solution was stirred 2 h at room temperature, then quenched with saturated $NaHCO_3$ solution. The aqueous phase was extracted 3X with EtOAc and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated to a white solid. Purification by flash chromatography (2X with EtOAc) afforded the title compound (145 mg, 51%) as a white solid.

(−) FAB-MS m/z 971.3 (M−), 590 (southern fragment), 379.1 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.60 (m, 1H, C(42)H), 3.98 (s, 2H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 172.1, 59.7.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 1.80 nM

LAF ratio: 0.83

Percent change in adjuvant arthritis versus control: −88%

EXAMPLE 3

Rapamycin 42-ester with 2.2-dimethyl-3-(tetrahydropyran-2-yloxy)propionic acid

To a solution of the 2,2-dimethyl-3-hydroxypropionic acid THP-ether (0.319 g, 1.58 mmol) and triethylamine (0.22 mL, 1.58 mmol) in 5 mL dry THF at 0 ° C. under nitrogen was added 2,4,6-trichlorobenzoyl chloride (0.25 mL, 1.58 mmol) dropwise via syringe. The mixture was stirred 4.5 h at room temperature. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and a warm water bath. The residue was dissolved in 5 mL benzene, then rapamycin (1.31 g, 1.43 mmol) and DMAP (0.193 g, 1.58 mmol) were added. The mixture was stirred overnight at room temperature, diluted with EtOAc, washed with 1N HCl (aq), saturated $NaHCO_3$ (aq), H_2O and brine, dried over $MgSO_4$, filtered and concentrated to a yellow oily solid. Flash chromatography (1X with 60% EtOAc-hexane, 1X with 55% EtOAc-hexane) afforded the title compound (0.356 g, 23%), as a white solid.

(−)FAB-MS m/z 1097.7 (M−), 590.4 (southern fragment), 505.3 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42H), 4.55 (m, 1H), 3.69 (m, 1H), 3.60 (m, 2H), 3.42 (m, 1H), 1.13 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 175.0, 98.0, 73.8, 60.7, 42.6, 30.0, 24.9, 22.0, 21.6, 18.7.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 7.10 nM

LAF ratio: 0.34

EXAMPLE 4

Rapamycin 42-ester with 3-hydroxy-2,2-dimethylpropionic acid

p-Toluenesulfonic acid (10 mg) was added to a solution of the product of Example 3 (250 mg, 0.23 mmol) in 10 mL CH_3OH at 0 ° C. The solution was stirred 2 hours at room temperature, then quenched with saturated $NaHCO_3$ solution. The aqueous phase was extracted 3X with EtOAc and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated to a white solid. Purification by flash chromatography (2X with 75% EtOAc-hexane) afforded the title compound (103 mg, 45%) as a white solid.

(−) FAB-MS m/z 1013.3 (M[31]), 590.2 (southern fragment), 421.1 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.48 (m, 1H, C(42)H), 3.39 (d, 2H), 106 (s, 6H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 175.5, 68.0, 44.1, 21.7.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}:0.80 nM

LAF ratio: 1.25

Skin graft survival time: 10.7±0.5 days

EXAMPLES 5 AND 6

Rapamycin 42-ester with 2,2-dimethyl[1,3]dioxalane-4-carboxylic acid (Ex. 5) Rapamycin 31,42-diester with 2,2-dimethyl[1.3]dioxalane-4-carboxylic acid (EX. 6)

2,4,6-Trichlorobenzoyl chloride (0.56 mL, 3.61 mmol) was added via syringe to a solution of the 2,3-dihydroxypropionic acid isopropylidene ketal (0.527 g, 3.61 mmol) and triethylamine (0.50 mL, 3.61 mmol) in 10 mL THF at 0 ° C. under nitrogen. The mixture was stirred 4 h at room temperature. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 15 mL benzene and rapamycin (3.00 g, 3.28 mmol), then DMAP (0.441 g, 3.61 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with cold 1N HCl (aq), saturated $NaHCO_3$ (aq) and brine, dried over $MgSO_4$, filtered and concentrated to a yellow foam. Flash chromatography on silica gel (gradient elution: 50–60–75–100% EtOAc-hexane, 4X with 65% EtOAc-hexane) afforded the title compounds. The less polar 31,42-diester (0.415 g) eluted first and the more polar 42-monoester (0.601 g, 16%) eluted second, and were isolated as white solids.

EXAMPLE 5

(−)FAB-MS m/z 1041.4 (M−), 590.3 (southern fragment), 449.2 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.6 (m, 1H, C(42)H), 4.6 (m, 1H), 4.20 (dd, 1H), 3.96 (m, 1H), 1.36 (s, 3H), 1.30 (s, 3H). ^{13}C

NMR (100.6 MHz, d-6 DMSO) δ 170.5, 110.2, 73.4, 66.6, 25.7, 25.4.

EXAMPLE 6

(−)FAB-MS m/z 1169.6 (M−). ^{1}H NMR (400 MHz, d-6 DMSO) δ 5.3 (m, 1H, C(31)H), 4.6 (m, 1H, C(42)H), 4.6 (m, 2H), 4.19 (t, 1H), 4.13 (t, 1H), 3.9 (m, 2H), 1.36 (s, 3H), 1.33 (s, 3H), 1.30 (s, 3H), 1.28 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 170.5, 169.2, 110.3, 110.2, 73.4, 66.6, 66.5, 25.8, 25.7, 25.4, 25.1.

Results obtained in standard pharmacological test procedures:

EXAMPLE 5

LAF IC_{50}: 1.20 nM
LAF ratio: 0.74

EXAMPLE 6

LAF IC_{50}: 1.30 nM
LAF ratio: 0.5

EXAMPLE 7

Rapamycin 42-ester with 2,3-dihydroxypropionic acid

A solution of the product of Example 5 (351 mg, 0.34 mmol) in 10 mL THF and 10 mL 1N HCl was stirred at room temperature for 6 h. The mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated to an oil. Flash chromatography (1X with EtOAc, 1X with 10% MeOH-CH_2Cl_2, 1X with 5% MeOH-EtOAc) afforded the title compound (78 mg, 23%) as a white solid.

(−)FAB-MS m/z 1001.2 (M−), 590.2 (southern fragment), 409.1 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 5 4.5 (m, 1H, C(42)H), 3.60 (m, 1H), 3.45 (m, 2H).

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 1.4 nM
LAF ratio: 0.40

EXAMPLE 8

Rapamycin 42-ester with 2.2-dimethyl[1.3dioxane-5-carboxylic acid

2,4,6-Trichlorobenzoyl chloride (0.98 mL, 6.27 mmol) was added via syringe to a solution of the 2-(hydroxymethyl)-3-hydroxypropionic acid isopropylidene ketal (1.000 g, 6.24 mmol) and triethylamine (0.90 mL, 6.46 mmol) in 20 mL THF at 0 ° C. under nitrogen. The mixture was stirred for 4 h at room temperature, and a white precipitate formed. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 20 mL benzene, then rapamycin (5.70 g, 6.24 mmol) and DMAP (0.762 g, 6.24 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated to a yellow solid. Flash chromatography (75% EtOAc-hexane) afforded the title compound (4.17 g, 63%) as a white solid.

(−)FAB-MS m/z 1055.8 (M−), 590.5 (southern fragment), 463.4 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42)H), 3.95 (m, 4H), 1.30 (s, 6H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 170.1, 97.4, 59.5, 24.8, 22.5.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 0.76 nM
LAF ratio: 0.45

EXAMPLE 9

Rapamycin 42-ester with 3-hydroxy-2-hydroxymethylpropionic acid

A solution of the product of Example 8 (3.30 g, 3.12 mmol) in 50 mL THF and 25 mL 1N HCl was stirred 2 h at room temperature. The solution was diluted with saturated $NaHCO_3$ solution and extracted with EtOAc (3X). The combined organic phases were washed with saturated NaCl (aq), dried over $MgSO_4$, filtered and concentrated to a yellow foam. Purification by flash chromatography (1X with EtOAc; 2X with 5% EtOH-EtOAc) afforded the title compound (1.68 g, 53 %) as a white solid.

(−)FAB-MS m/z 1015.5 (M−), 590.3 (southern fragment), 423.3 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.6 (br s, 2H), 4.55 (m, 1H, C(42)H), 3.55 (m, 4H), 2.57–2.53 (m, 1H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 172.2, 59.3, 51.5.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 0.84 nM
LAF ratio: 0.57

EXAMPLE 10

Rapamycin 42-ester with 2,2,5-trimethyl[1.3dioxane-5-carboxylic acid

To a solution of the 2,2-bis(hydroxymethyl)propionic acid isopropylidene ketal (1.041 g, 5.98 mmol) (prepared according to the procedure of Bruice, J. Am. Chem. Soc. 89:3568 (1967)) and triethylamine (0.83 mL, 5.98 mmol) in 20 mL anhydrous THF at 0 ° C. under nitrogen was added 2,4,6-trichlorobenzoyl chloride (0.93 mL, 5.98 mmol) and the resultant white suspension was stirred 5 h at room temperature. The precipitate was removed by vacuum filtration, rinsing the flask and filter cake with an additional 10 mL dry THF. The filtrate was concentrated by rotary evaporation to a white solid. The residue was dissolved in 20 mL dry benzene, then rapamycin (5.47 g, 5.98 mmol) and DMAP (0.731 g, 5.98 retool) were added. After stirring overnight at room temperature, the mixture was diluted with EtOAc, washed with H_2O and saturated NaCl (aq), dried over $MgSO_4$, filtered and evaporated to a yellow oil. Flash chromatography (5X with 60% EtOAc-hexane) afforded the title compound (2.2 g, 34%) as a white solid.

(−)FAB-MS m/z 1069.5 (M−), 590.3 (southern fragment), 477.2 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.57 (m, 1H, C(42)H, 4.02 (d, 2H), 3.60 (d, 2H), 1.34 (s, 3H), 1.24 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 173.2, 99.0, 65.0, 22.2, 18.1.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 4.90 nM
LAF ratio: 0.41
Skin graft survival time: 11.0±1.3 days

EXAMPLE 11

Rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid

A solution of the product of Example 10 (2.8 g, 2.65 mmol) in 50 mL THF and 25 mL 1N HCl was stirred at room temperature for 4 h. The mixture was diluted with water and extracted three times with EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ solution, saturated NaCl solution, dried over $MgSO_4$, filtered and evaporated to a yellow oily solid. Purification by flash chromatography (3X with EtOAc) afforded the title compound (1.6 g, 59%).

(−)FAB-MS m/z 1029.6 (M−), 590.4 (southern fragment), 437.3 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.5 (m, 1H, C(42)H), 3.45 (s, 4H), 1.04 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 174.2, 63.7, 63.6, 49.9, 16.8.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 0.80 and 1.80 nM

LAF ratio: 1.00 and 0.44

Skin graft survival time: 11.4±1.5 and 12.0±1.1 days

Percent change in adjuvant arthritis versus control: −88%

EXAMPLE 12

Rapamycin 42-ester with 2,2-dimethyl-5-(2-trimethylsilanylethoxymethyl)[1,3]dioxane-5-carboxylic acid

2,4,6-Trichlorobenzoyl chloride (0.14 mL, 0.86 mmol) was added via syringe to a solution of the 2,2-bis(hydroxymethyl)-2-(2-trimethylsilylethoxy)propionic acid isopropylidene ketal (0.250 g, 0.86 mmol) and triethylamine (0.12 mL, 0.86 mmol) in 2 mL THF at 0° C. under nitrogen. The mixture was stirred for 4 h at room temperature, and a white precipitate formed. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 2 mL benzene, then rapamycin (0.786 g, 0.86 mmol) and DMAP (0.105 g, 0.86 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated to a yellow solid. Flash chromatography (gradient elution: 40-60-80-100% EtOAc-hexane) afforded the title compound (0.559 g, 54%) as a white solid.

(−)FAB-MS m/z 1185.2 (M−), 590.1 (southern fragment), 593 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42)H), 3.73 (m, 4H), 3.57 (s, 2 H), 3.43 (t, 2H), 1.29 (s, 6H), 0.79 (t, 2H), −0.04 (s, 9H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 171.1, 97.7, 70.2, 68.1, 61.3, 46.0, 24.6, 22.1, 14.6, −1.3.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 7.20 nM

LAF ratio: 0.05

EXAMPLES 13 and 14

Rapamycin 42-ester with 3-methyl-1,5-dioxa-spiro[5.5]undecane 3-carboxylic acid (Ex. 13)

Rapamycin 31.42-diester with 3-methyl-1.5-dioxa-spiro[5.5]undecane 3-carboxylic acid (Ex. 14)

2,4,6-Trichlorobenzoyl chloride (0.16 mL, 1.0 mmol) was added via syringe to a solution of the 2,3-dihydroxypropionic acid cyclohexylidene ketal (0.214 g, 1.0 mmol) and triethylamine (0.14 mL, 1.0 mmol) in 2.5 mL THF at 0° C. under nitrogen. The mixture was stirred 4 h at room temperature. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 3 mL benzene and rapamycin (0.457 g, 0.5 mmol), then DMAP (0.061 g, 0.5 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with cold 1N HCl (aq), saturated $NaHCO_3$ (aq) and brine, dried over $MgSO_4$, filtered and concentrated to a yellow foam. Flash chromatography on silica gel (45-50% EtOAc-hexane) afforded the title compounds. The 31,42-diester (0.168 g, 26%) eluted first and the more polar 42-monoester (0.301 g, 52%) eluted second, and the products were isolated as white solids.

EXAMPLE 13

(−)FAB-MS m/z 1109.5 (M−), 590.3 (southern fragment), 517.3 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42)H), 3.61 (t, 4H), 1.04 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 173.3, 97.2, 64.2.

EXAMPLE 14

(−)FAB-MS m/z 1305.6 (M−). ^{1}H NMR (400 MHz, d-6 DMSO) δ 5.25 (m, 1H, C(31)H), 4.55 (m, 1H, C(42)H), 3.64-3.54 (m, 8H), 1.05 (s, 3H), 0.97 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 173.2, 172.1, 97.3, 97.2, 64.3, 64.2, 63.9.

Results obtained in standard pharmacological test procedures:

EXAMPLE 13

LAF IC_{50}: 0.6 nM

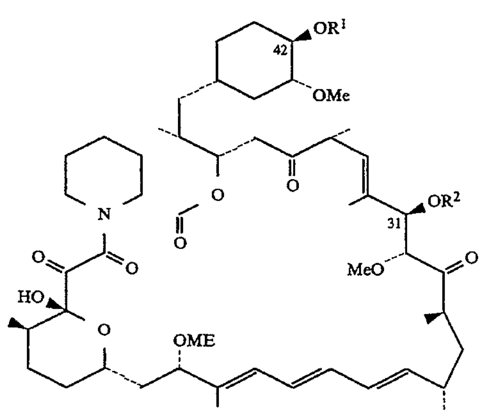
LAF ratio: 2.00

EXAMPLE 14

LAF: inhibited T-cell proliferation by 43% at 0.1 μM

What is claimed is:

1. A compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, alkenyl of 2-7 carbon atoms,

alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$—F, or$—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

b=0–6;

d=0–6 ; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein R^2 is hydrogen or a pharmaceutically acceptable salt thereof.

3. The compound of claim 2, wherein b=0 and d=0 or a pharmaceutically acceptable salt thereof.

4. The compound of claim 3, wherein R^8 and R^9 are each, independently hydrogen, alkyl, or $—(CR^3R^4)_fOR^{10}$, or are taken together to form X or a pharmaceutically acceptable salt thereof.

5. The compound of claim 1 which is rapamycin 42-ester with (tetrahydropyran-2-yloxy)acetic acid or a pharmaceutically acceptable salt thereof.

6. The compound of claim 1 which is rapamycin 42-ester with hydroxyacetic acid or a pharmaceutically acceptable salt thereof.

7. The compound of claim 1 which is rapamycin 42-ester with 2,2-dimethyl-3-(tetrahydropyran-2-yloxy)propionic acid or a pharmaceutically acceptable salt thereof.

8. The compound of claim 1 which is rapamycin 42-ester with 3-hydroxy-2,2-dimethylpropionic acid or a pharmaceutically acceptable salt thereof.

9. The compound of claim 1 which is rapamycin 42-ester with 2,2-dimethyl[1,3]dioxalane-4-carboxylic acid or a pharmaceutically acceptable salt thereof.

10. The compound of claim 1 which is rapamycin 31,42-diester with 2,2-dimethyl[1,3]dioxalane-4-carboxylic acid or a pharmaceutically acceptable salt thereof.

11. The compound of claim 1 which is rapamycin 42-ester with 2,3-dihydroxypropionic acid or a pharmaceutically acceptable salt thereof.

12. The compound of claim 1 which is rapamycin 42-ester with 2,2-dimethyl[1,3]dioxane-5-carboxylic acid or a pharmaceutically acceptable salt thereof.

13. The compound of claim 1 which is rapamycin 42-ester with 3-hydroxy-2-hydroxymethylpropionic acid or a pharmaceutically acceptable salt thereof.

14. The compound of claim 1 which is rapamycin 42-ester with 2,2,5-trimethyl[1,3]dioxane-5-carboxylic acid or a pharmaceutically acceptable salt thereof.

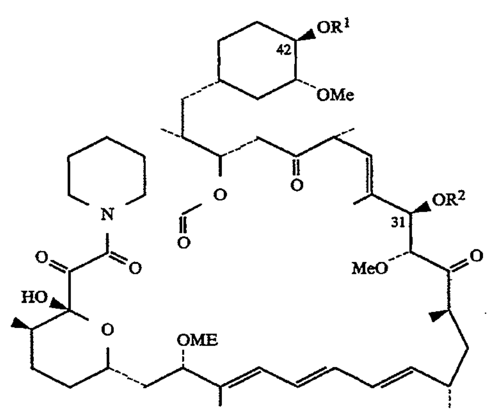
15. The compound of claim 1 which is rapamycin 42-ester with 2,2-bis(hydroxymethyl)propionic acid or a pharmaceutically acceptable salt thereof.

16. The compound of claim 1 which is rapamycin 42-ester with 2,2-dimethyl-5-(2-trimethylsilanylethoxymethyl)[1,3]-dioxane-5-carboxylic acid or a pharmaceutically acceptable salt thereof.

17. The compound of claim 1 which is rapamycin 42-ester with 3-methyl-1,5-dioxa-spiro[5.5]undecane 3-carboxylic acid or a pharmaceutically acceptable salt thereof.

18. The compound of claim 1 which is rapamycin 31,42-diester with 3-methyl-1,5-dioxa-spiro[5.5]undecane 3-carboxylic acid or a pharmaceutically acceptable salt thereof.

19. A method of treating transplantation rejection or graft vs. host disease in a mammal in need thereof, which comprises administering to said mammal an antirejection effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of

3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, —F, or $-CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, —F, or $-CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

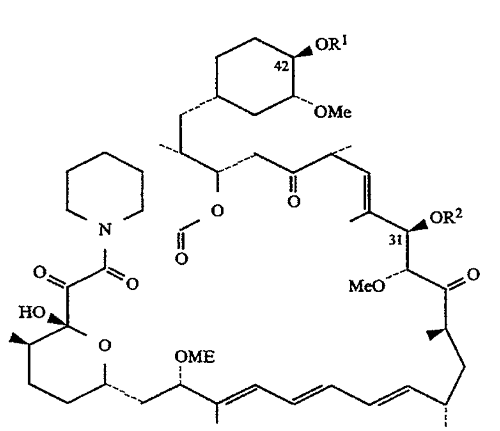
b=0–6;

d=0–6 ; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $-(CR^3R^4)_fOR^{10}$, X, or $-(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

20. A method of treating a fungal infection in a mammal in need thereof, which comprises administering to said mammal an antifungal effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $-CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^89^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, —F, or $-CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, —F, or $-CO_2R_{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, —F, or $-CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

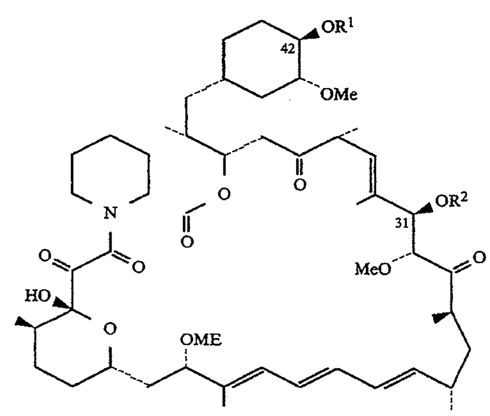
b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $-(CR^3R^4)_fOR^{10}$, X, or $-(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

21. A method of treating rheumatoid arthritis in a mammal in need thereof, which comprises administering to said mammal an antiarthritis effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $-CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms,

alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

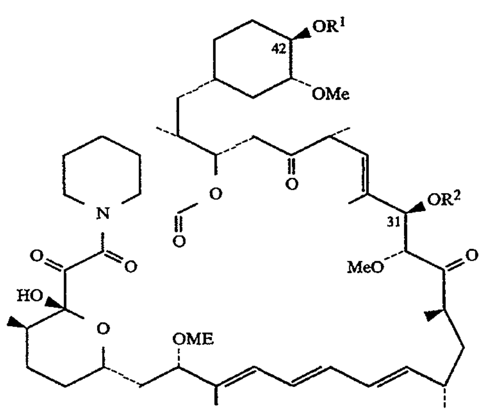
b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

22. A method of treating restenosis in a mammal in need thereof, which comprises administering to said mammal an antiproliferative effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

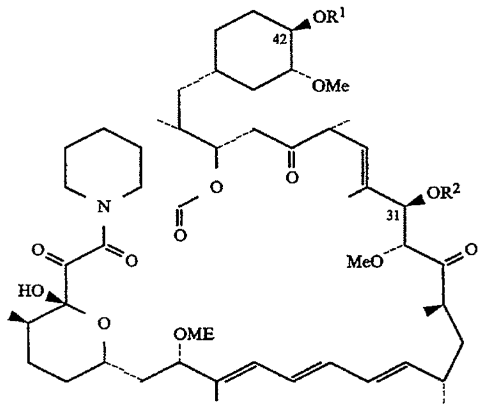
b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

23. A method of treating pulmonary inflammation in a mammal in need thereof, which comprises administering to said mammal an antiinflammatory effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $-CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or $-F$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, $-F$, or $-CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, $-F$, or $-CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, $-F$, or $-CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

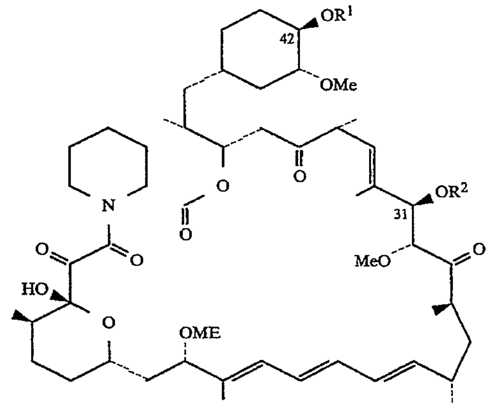
$b=0–6$;

$d=0–6$; and

$f=0–6$

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $-(CR^3R^4)_fOR^{10}$, X, or $-(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

24. A pharmaceutical composition which comprises a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $-CO(CR^3R^4)_bCR^5R^6)_dCR^7R^8R^9$; R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or $-F$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, $-F$, or $-CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3$, $-F$, or $-CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $-(CR^3R^4)_fOR^{10}$, $-CF_3-F$, or $-CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $-(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

$b=0–6$;

$d=0–6$; and

$f=0–6$

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $-(CR^3R^4)_fOR^{10}$, X, or $-(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

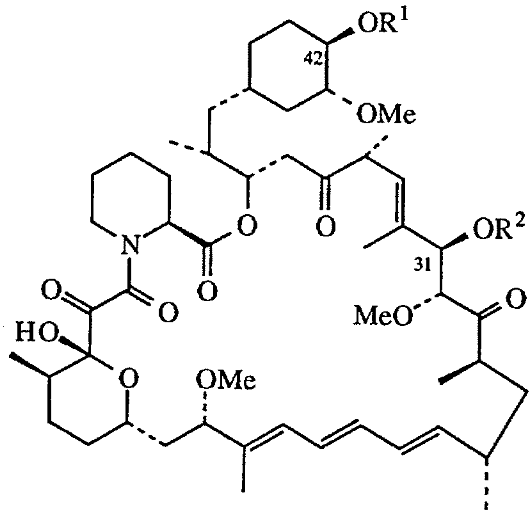
PATENT NO. : 5,362,718

DATED : November 8, 1994

INVENTORS : Jerauld S. Skotnicki, Christina L. Leone, Guy A. Schiehser

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On Page 2 of the Abstract, first column, and in the Patent column 2, lines 1-19; column 14, lines 45-64; column 16, lines 38-57; column 17, lines 41-60; column 18, lines 45-64; column 19, lines 48-67; column 21, lines 1-19; and column 22, lines 3-22, please delete the structure and insert therefor:

--  --

Signed and Sealed this

Twelfth Day of August, 1997

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

US005464650A

United States Patent [19]

Berg et al.

[11] Patent Number: 5,464,650

[45] Date of Patent: Nov. 7, 1995

[54] **INTRAVASCULAR STENT AND METHOD**

[75] Inventors: **Eric P. Berg**; **Ronald J. Tuch**, both of Plymouth; **Michael Dror**, Edina; **Rodney G. Wolff**, Minnetonka Beach, all of Minn.

[73] Assignee: **Medtronic, Inc.**, Minneapolis, Minn.

[21] Appl. No.: **52,878**

[22] Filed: **Apr. 26, 1993**

[51] **Int. Cl.**^{6} **B05D 1/02**; B05D 1/18; B05D 1/38

[52] **U.S. Cl.** **427/2.30**; 427/2.25; 427/2.28

[58] **Field of Search** 427/2, 421, 2.12, 427/2.13, 2.28, 2.30, 430.1, 2.25

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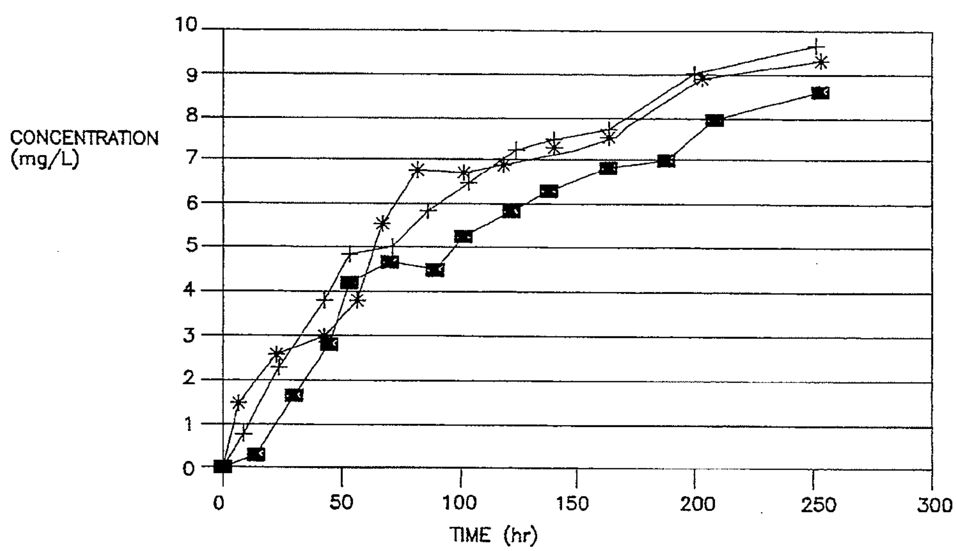
"Restonosis After Balloon Angioplasty" by Schwartz, et al., in Circulation, vol. 82, No. 6, pp. 2190–2198 Dec. 1990.

Primary Examiner—Diana Dudash
Attorney, Agent, or Firm—Daniel W. Latham; Harold R. Patton

[57] **ABSTRACT**

A method for making an intravascular stent by applying to the body of a stent a solution which includes a solvent, a polymer dissolved in the solvent and a therapeutic substance dispersed in the solvent and then evaporating the solvent. The inclusion of a polymer in intimate contact with a drug on the stent allows the drug to be retained on the stent during expansion of the stent and also controls the administration of drug following implantation. The adhesion of the coating and the rate at which the drug is delivered can be controlled by the selection of an appropriate bioabsorbable or biostable polymer and the ratio of drug to polymer in the solution. By this method, drugs such as dexamethasone can be applied to a stent, retained on a stent during expansion of the stent and elute at a controlled rate.

22 Claims, 2 Drawing Sheets



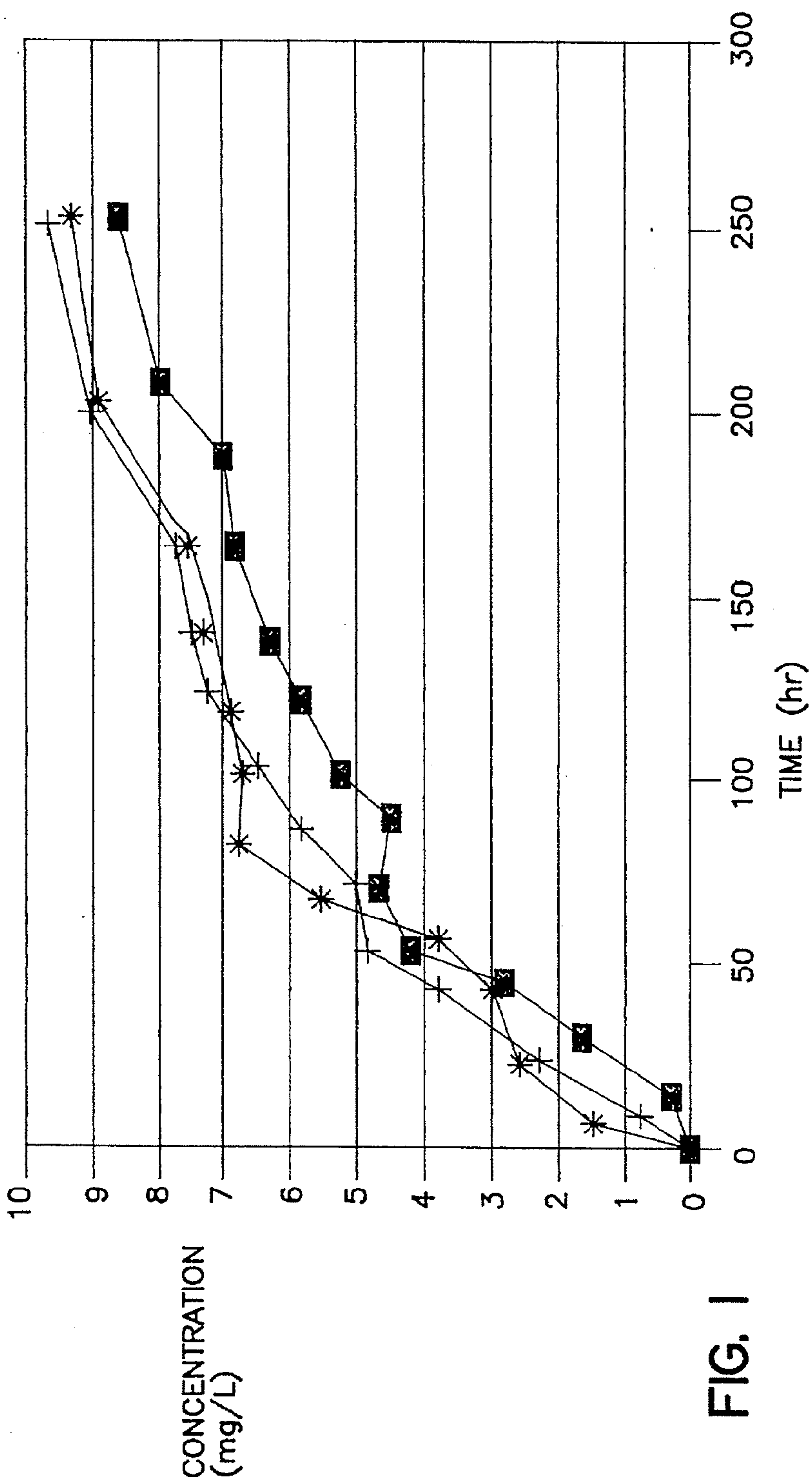

CONCENTRATION
(mg/L)
10
9
8
7
6
5
4
3
2
1
0
TIME (hr)
0
50
100
150
200
250
300

FIG. I

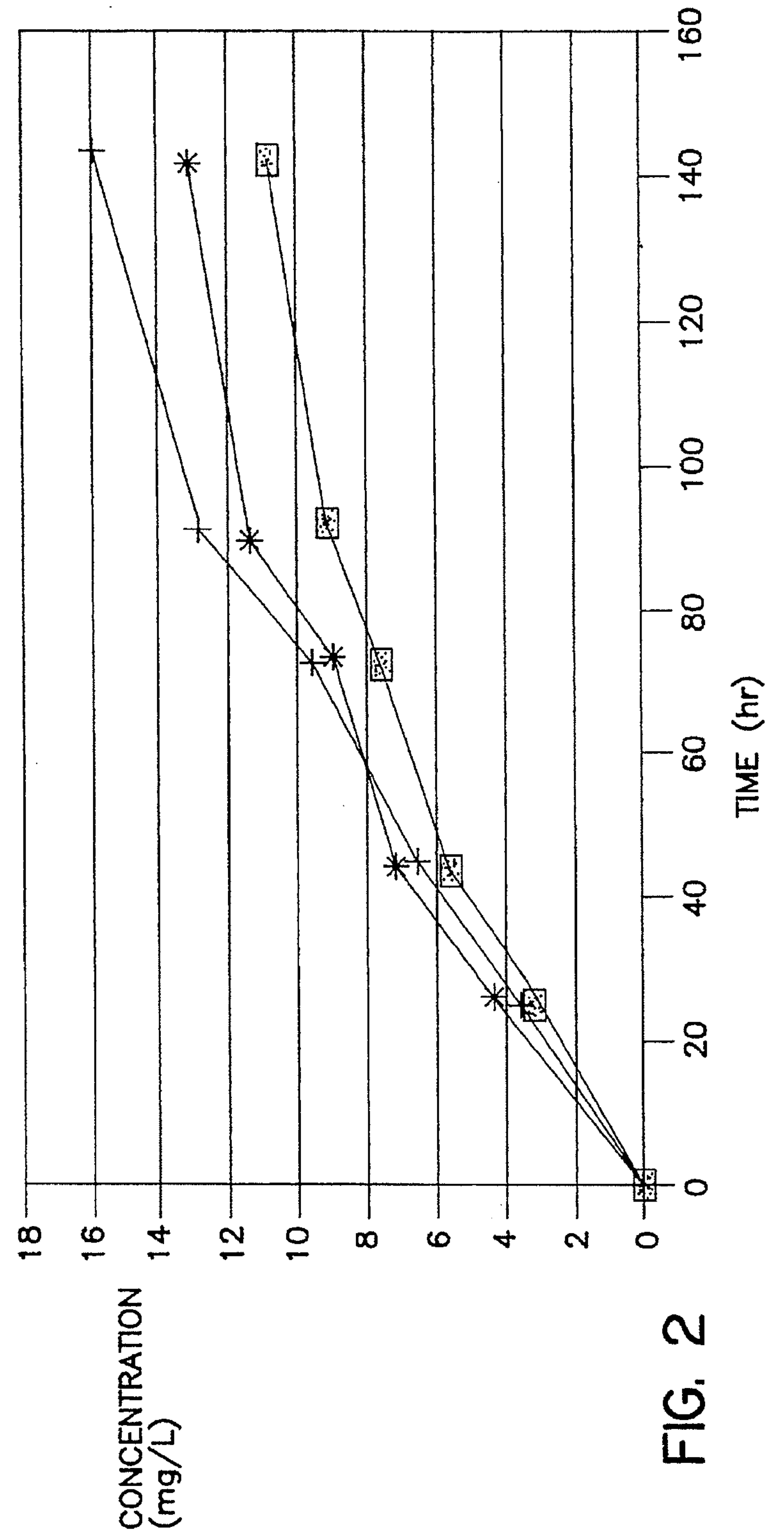

CONCENTRATION (mg/L)
0
2
4
6
8
10
12
14
16
18
TIME (hr)
0
20
40
60
80
100
120
140
160

FIG. 2

INTRAVASCULAR STENT AND METHOD

BACKGROUND OF THE INVENTION

This invention relates to intravascular stents for treatment of injuries to blood vessels and particularly to stents having a framework onto which a therapeutic substance or drug is applied.

Although angioplasty procedures have increased greatly in popularity for treatment of occluded arteries, the problem of restenosis following the angioplasty treatment remains a significant problem. Restenosis is the closure of a peripheral or coronary artery following trauma to the artery caused by efforts to open an occluded portion of the artery by angioplasty, such as, for example, by balloon dilation, atherectomy or laser ablation treatment of the artery. For these angioplasty procedures, restenosis occurs at a rate of about 30–60% depending upon the vessel location, lesion length and a number of other variables.

One aspect of restenosis may be simply mechanical; e.g. caused by the elastic rebound of the arterial wall and/or by dissections in the vessel wall caused by the angioplasty procedure. These mechanical problems have been successfully addressed by the use of stents to tack-up dissections and prevent elastic rebound of the vessel, thereby reducing the level of restenosis for many patients. The stent is typically inserted by catheter into a vascular lumen and expanded into contact with the diseased portion of the arterial wall, thereby providing internal support for the lumen. Examples of stents which have been successfully applied over a PTCA balloon and radially expanded at the same time as the balloon expansion of an affected artery include the stents disclosed in U.S. Pat. No. 4,733,665 issued to Palmaz, U.S. Pat. No. 4,800,882 issued to Gianturco and U.S. Pat. No. 4,886,062 issued to Wiktor which are incorporated herein by reference in their entirety.

Another aspect of restenosis is believed to be a natural healing reaction to the injury of the arterial wall that is caused by angioplasty procedures. The final result of the complex steps of the healing process is intimal hyperplasia, the migration and proliferation of medial smooth muscle cells, until the artery is again occluded.

To address both aspects of the restenosis problem, it has been proposed to provide stents which are seeded with endothelial cells (Dichek,D. A. et al Seeding of Intravascular Stents With Genetically Engineered Endothelial Cells; Circulation 1989; 80: 1347–1353). In that experiment, sheep endothelial cells that had undergone retrovirus-mediated gene transfer for either bacterial beta-galactosidase or human tissue-type plasminogen activator were seeded onto stainless steel stents and grown until the stents were covered. The cells were therefore able to be delivered to the vascular wall where they could provide therapeutic proteins. Other methods of providing therapeutic substances to the vascular wall include simple heparin-coated metallic stents, whereby a heparin coating is ionically or covalently bonded to the stent. Still other methods of providing therapeutic substances to the vascular wall by means of stents have also been proposed such as in U.S. Pat. No. 5,102,417 issued to Palmaz or in international patent application WO 91/12779 "Intraluminal Drug Eluting Prosthesis" and international patent application WO 90/13332 "Stent With Sustained Drug Delivery". In those applications, it is suggested that antiplatelet agents, anticoagulant agents, antimicrobial agents, antimetabolic agents and other drugs could be supplied in stents to reduce the incidence of restenosis.

Metal stents such as those disclosed in U.S. Pat. No. 4,733,665 issued to Palmaz, U.S. Pat. No. 4,800,882 issued to Gianturco or U.S. Pat. No. 4,886,062 issued to Wiktor could be suitable for drug delivery in that they are capable of maintaining intimate contact between a substance applied to the outer surface of the stent and the tissues of the vessel to be treated. However, there are significant problems to be overcome in order to secure a therapeutically significant amount of a substance onto the metal of the stent; to keep it on the stent during expansion of the stent into contact with the blood vessel wall; and also controlling the rate of drug delivery from the drug on the stent to the vessel wall.

It is therefore an object of the present invention to provide a stent having a therapeutically significant amount of a drug applied thereto.

It is also an object of the present invention to provide a stent which may be delivered and expanded in a selected blood vessel without losing a therapeutically significant amount of a drug applied thereto.

It is also an object of the present invention to provide a drug-containing stent which allows for a sustained release of the drug to vascular tissue.

It is also an object of the present invention to provide a simple method for applying to a stent a coating of a therapeutic substance.

SUMMARY OF THE INVENTION

These and other objects are accomplished by the present invention. We have discovered a method for making an intravascular stent by applying to the body of a stent, and in particular to its tissue-contacting surface, a solution which includes a solvent, a polymer dissolved in the solvent and a therapeutic substance dispersed in the solvent and then evaporating the solvent. The inclusion of a polymer in intimate contact with a drug on the stent allows the drug to be retained on the stent in a resilient matrix during expansion of the stent and also slows the administration of drug following implantation. The method can be applied whether the stent has a metallic or polymeric surface. The method is also an extremely simple method since it can be applied by simply immersing the stent into the solution or by spraying the solution onto the stent. The amount of drug to be included on the stent can be readily controlled by applying multiple thin coats of the solution while allowing it to dry between coats. The overall coating should be thin enough so that it will not significantly increase the profile of the stent for intravascular delivery by catheter. It is therefore preferably less than about 0.002 inch thick and most preferably less than 0.001 inch thick. The adhesion of the coating and the rate at which the drug is delivered can be controlled by the selection of an appropriate bioabsorbable or biostable polymer and by the ratio of drug to polymer in the solution. By this method, drugs such as glucocorticoids (e.g. dexamethasone, betamethasone), heparin, hirudin, tocopherol, angiopeptin, aspirin, ACE inhibitors, growth factors, oligonucleotides, and, more generally, antiplatelet agents, anticoagulant agents, antimitotic agents, antioxidants, antimetabolite agents, and anti-inflammatory agents can be applied to a stent, retained on a stent during expansion of the stent and elute the drug at a controlled rate. The release rate can be further controlled by varying the ratio of drug to polymer in the multiple layers. For example, a higher drug-to-polymer ratio in the outer layers than in the inner layers would result in a higher early dose which would decrease over time.

In operation, the stent made according to the present invention can deliver drugs to a body lumen by introducing the stent transluminally into a selected portion of the body lumen and radially expanding the stent into contact with the body lumen. The transluminal delivery can be accomplished by a catheter designed for the delivery of stents and the radial expansion can be accomplished by balloon expansion of the stent, by self-expansion of the stent, or a combination of self-expansion and balloon expansion.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a plot showing elution profiles for stents according to the present invention with a coating of dexamethasone and poly(L-lactic acid) made according to Example 6.

FIG. 2 is a plot showing elution profiles for sterilized stents according to the present invention with a coating of dexamethasone and poly(L-lactic acid) made according to Example 7.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for making an intravascular stent. The underlying structure of the stent can be virtually any stent design, whether of the self-expanding type or of the balloon-expandable type and whether metal or polymeric. Thus metal stent designs such as those disclosed in U.S. Pat. No. 4,733,665 issued to Palmaz, U.S. Pat. No. 4,800,882 issued to Gianturco or U.S. Pat. No. 4,886,062 issued to Wiktor could be used in the present invention. The stent could be made of virtually any bio-compatible material having physical properties suitable for the design. For example, tantalum and stainless steel have been proven suitable for many such designs and could be used in the present invention. Also, stents made with biostable or bioabsorbable polymers such as poly(ethylene terephthalate), polyacetal, poly(lactic acid), poly(ethylene oxide)/poly(butylene terephthalate) copolymer could be used in the present invention. Although the stent surface should be clean and free from contaminants that may be introduced during manufacturing, the stent surface requires no particular surface treatment in order to retain the coating applied in the present invention. Both the inner and outer surfaces of the stent may be provided with the coating according to the present invention.

In order to provide the coated stent according to the present invention, a solution which includes a solvent, a polymer dissolved in the solvent and a therapeutic substance dispersed in the solvent is first prepared. It is important to choose a solvent, a polymer and a therapeutic substance that are mutually compatible. It is essential that the solvent is capable of placing the polymer into solution at the concentration desired in the solution. It is also essential that the solvent and polymer chosen do not chemically alter the therapeutic character of the therapeutic substance. However, the therapeutic substance only needs to be dispersed throughout the solvent so that it may be either in a true solution with the solvent or dispersed in fine particles in the solvent. Examples of some suitable combinations of polymer, solvent and therapeutic substance are set forth in Table 1 below.

TABLE 1

POLYMER	SOLVENT	THERAPEUTIC SUBSTANCE
poly(L-lactic acid)	chloroform	dexamethasone
poly(lactic acid-co-glycolic acid)	acetone	dexamethasone
polyether urethane	N-methyl pyrrolidone	tocopherol (vitamin E)
silicone adhesive	xylene	dexamethasone phosphate
poly(hydroxy-butyrate-co-hydroxyvalerate)	dichloro-methane	aspirin
fibrin	water (buffered saline)	heparin

The solution is applied to the stent and the solvent is allowed to evaporate, thereby leaving on the stent surface a coating of the polymer and the therapeutic substance. Typically, the solution can be applied to the stent by either spraying the solution onto the stent or immersing the stent in the solution. Whether one chooses application by immersion or application by spraying depends principally on the viscosity and surface tension of the solution, however, it has been found that spraying in a fine spray such as that available from an airbrush will provide a coating with the greatest uniformity and will provide the greatest control over the amount of coating material to be applied to the stent. In either a coating applied by spraying or by immersion, multiple application steps are generally desirable to provide improved coating uniformity and improved control over the amount of therapeutic substance to be applied to the stent.

The polymer chosen must be a polymer that is biocompatible and minimizes irritation to the vessel wall when the stent is implanted. The polymer may be either a biostable or a bioabsorbable polymer depending on the desired rate of release or the desired degree of polymer stability, but a bioabsorbable polymer is probably more desirable since, unlike a biostable polymer, it will not be present long after implantation to cause any adverse, chronic local response. Bioabsorbable polymers that could be used include poly(L-lactic acid), polycaprolactone, poly(lactide-co-glycolide), poly(hydroxybutyrate), poly(hydroxybutyrate-co-valerate), polydioxanone, polyorthoester, polyanhydride, poly(glycolic acid), poly(D,L-lactic acid), poly(glycolic acid-co-trimethylene carbonate), polyphosphoester, polyphosphoester urethane, poly(amino acids), cyanoacrylates, poly(trimethylene carbonate), poly(iminocarbonate), copoly(ether-esters) (e.g. PEO/PLA), polyalkylene oxalates, polyphosphazenes and biomolecules such as fibrin, fibrinogen, cellulose, starch, collagen and hyaluronic acid. Also, biostable polymers with a relatively low chronic tissue response such as polyurethanes, silicones, and polyesters could be used and other polymers could also be used if they can be dissolved and cured or polymerized on the stent such as polyolefins, polyisobutylene and ethylene-alphaolefin copolymers; acrylic polymers and copolymers, vinyl halide polymers and copolymers, such as polyvinyl chloride; polyvinyl ethers, such as polyvinyl methyl ether; polyvinylidene halides, such as polyvinylidene fluoride and polyvinylidene chloride; polyacrylonitrile, polyvinyl ketones; polyvinyl aromatics, such as polystyrene, polyvinyl esters, such as polyvinyl acetate; copolymers of vinyl monomers with each other and olefins, such as ethylene-methyl methacrylate copolymers, acrylonitrile-styrene copolymers, ABS resins,

and ethylene-vinyl acetate copolymers; polyamides, such as Nylon 66 and polycaprolactam; alkyd resins; polycarbonates; polyoxymethylenes; polyimides; polyethers; epoxy resins, polyurethanes; rayon; rayon-triacetate; cellulose, cellulose acetate, cellulose butyrate; cellulose acetate butyrate; cellophane; cellulose nitrate; cellulose propionate; cellulose ethers; and carboxymethyl cellulose.

The ratio of therapeutic substance to polymer in the solution will depend on the efficacy of the polymer in securing the therapeutic substance onto the stent and the rate at which the coating is to release the therapeutic substance to the tissue of the blood vessel. More polymer may be needed if it has relatively poor efficacy in retaining the therapeutic substance on the stent and more polymer may be needed in order to provide an elution matrix that limits the elution of a very soluble therapeutic substance. A wide ratio of therapeutic substance to polymer could therefore be appropriate and could range from about 10:1 to about 1:100.

The therapeutic substance used in the present invention could be virtually any therapeutic substance which possesses desirable therapeutic characteristics for application to a blood vessel. This can include both solid substances and liquid substances. For example, glucocorticoids (e.g. dexamethasone, betamethasone), heparin, hirudin, tocopherol, angiopeptin, aspirin, ACE inhibitors, growth factors, oligonucleotides, and, more generally, antiplatelet agents, anticoagulant agents, antimitotic agents, antioxidants, antimetabolite agents, and anti-inflammatory agents could be used. Antiplatelet agents can include drugs such as aspirin and dipyridamole. Aspirin is classified as an analgesic, antipyretic, anti-inflammatory and antiplatelet drug. Dypridimole is a drug similar to aspirin in that it has anti-platelet characteristics. Dypridimole is also classified as a coronary vasodilator. Anticoagulant agents can include drugs such as heparin, coumadin, protamine, hirudin and tick anticoagulant protein. Antimitotic agents and antimetabolite agents can include drugs such as methotrexate, azathioprine, vincristine, vinblastine, fluorouracil, adriamycin and mutamycin.

The following examples are exemplary of various aspects of the invention.

EXAMPLE 1

A 1% solution of dexamethasone in acetone was made, forming a clear solution. The solution was placed in an airbrush reservoir (Badger #200). Wiktor type tantalum wire stents were sprayed with the solution in short bursts while rotating the stents. The acetone quickly evaporated from the stents, leaving a white residue on the stent wire. The process was continued until all of the stent wires were coated. The drug elution rate for the stent was determined by immersing the stent in phosphate buffered saline solution (pH=7.4). Traces of dexamethasone were observed to remain on the immersed stents for less than 31 hours.

EXAMPLE 2

A 2% solution of dexamethasone in acetone was made, forming a solution with suspended particles of dexamethasone. The solution was placed into a tube. Wiktor type tantalum wire stents were dipped rapidly and were allowed to dry. Each stent was dipped into the solution 12–15 times to provide a white surface coating. Two stents were placed on an angioplasty balloon and were inflated on the balloon. Approximately 80% of the dexamethasone coating flaked off of the stents.

EXAMPLE 3

A solution of 1% dexamethasone and 0.5% poly(caprolactone) (Aldrich 18,160-9) in acetone was made. The solution was placed into a tube. Wiktor type tantalum wire stents were dipped rapidly and were allowed to dry. Each stent was dipped into the solution 12–15 times to provide a white surface coating. A stent so coated was expanded on a 3.5 mm angioplasty balloon causing a significant amount of the coating to become detached.

EXAMPLE 4

A solution of 1% dexamethasone and 0.5% poly(L-lactic acid) (Medisorb) in acetone was made. The solution was placed into a tube. Wiktor type tantalum wire stents were dipped rapidly and were allowed to dry. Each stent was dipped into the solution 12–15 times to provide a white surface coating. A stent so coated was expanded on a 3.5 mm angioplasty balloon causing only a small portion of the coating (less than 25%) to become detached)

EXAMPLE 5

A solution including a 2% dispersion of dexamethasone and a 1% solution of poly(L-lactic acid) (CCA Biochem MW=550,000) in chloroform was made. The solution was placed into an airbrush (Badger). Wiktor type tantalum wire stents were sprayed in short bursts and were allowed to dry. Each stent was sprayed with the solution about 20 times to provide a white surface coating. A stent so coated was expanded on a 3.5 mm angioplasty balloon. The coating remained attached to the stent throughout the procedure.

EXAMPLE 6

A solution including a 2% dispersion of dexamethasone and a 1% solution of poly(L-lactic acid) (CCA Biochem MW=550,000) in chloroform was made. The solution was placed into an airbrush (Badger #250-2). Wiktor type tantalum wire stents were suspended from a fixture and sprayed in 24 short bursts (6 bursts from each of the four directions perpendicular to the stent axis) and were allowed to dry. The resulting stents had a coating weight of about 0.0006–0.0015 grams. Three of the stents were tested for long term elution by placing one stent in 3.0 ml of phosphate buffered saline solution (pH=7.4) at room temperature without stirring. The amount of dexamethasone eluted was evaluated by measuring absorbance at 244 nm in a UV-VIS spectrophotometer. The results of this test are given in FIG. 1.

EXAMPLE 7

A solution including a 2% dispersion of dexamethasone and a 1% solution of poly(L-lactic acid) (Medisorb 100-L) in chloroform was made along with a control solution of 1% of poly(L-lactic acid) (Medisorb 100-L) in chloroform. The solutions was placed into an airbrush (Badger #250-2). Wiktor type tantalum wire stents were expanded on a 3.0 mm balloon, suspended from a fixture and sprayed in 16 short bursts (2–3 bursts of about 1 second followed by several minutes drying time between applications). The resulting dexamethasone-coated stents had an average coating weight of about 0.0012 grams while the polymer-coated stents had an average polymer weight of about 0.0004 grams. The stents were sterilized in ethylene oxide. Three of the sterilized dexamethasone-coated stents were tested for long term elution by placing one stent in 3.0 ml of phosphate buffered saline solution (pH=7.4) at room temperature with-

out stirring. The amount of dexamethasone eluted was evaluated by measuring absorbance at 244 nm in a UV-VIS spectrophotometer. The results of this test are given in FIG. 2. Dexamethasone-coated stents and polymer-coated control stents were implanted in the coronary arteries of 8 pigs (N=12 for each type) according to the method set forth in "Restenosis After Balloon Angioplasty—A Practical Proliferative Model in Porcine Coronary Arteries," by Robert S. Schwartz, et al, Circulation 82(6):2190–2200, Dec. 1990, and "Restenosis and the Proportional Neointimal Response to Coronary Artery Injury: Results in a Porcine Model" by Robert S. Schwartz et al, J Am Coll Cardiol; 19;267–74 Feb. 1992 with the result that when compared with the controls, the dexamethasone-coated stents reduced the amount of proliferation associated with the arterial injury.

It will be appreciated by those skilled in the art that while the invention has been described above in connection with particular embodiments and examples, the invention is not necessarily so limited and that numerous other embodiments, examples, uses, modifications and departures from the embodiments, examples and uses may be made without departing from the inventive concepts.

We claim:

1. A method for making an intravascular stent comprising the steps of:

(a) providing a cylindrical, radially expandable stent body;

(b) applying to the stent body by spraying a solution which includes a solvent, a polymer dissolved in the solvent and a therapeutic substance dispersed in the solvent;

(c) evaporating the solvent;

(d) repeating application and evaporating steps (b) and (c) to provide an amount of polymer and therapeutic substance on the stent body; and

(e) radially expanding the stent body and applied polymer and therapeutic substance such that the polymer and therapeutic substance are retained on the stent body.

2. A method according to claim 1, wherein the stent body has a metal surface.

3. A method according to claim 1, wherein the stent body has a polymeric surface.

4. A method according to claim 1 wherein the ratio of drug to dissolved polymer in the solution is varied in some of the application steps.

5. A method according to claim 1 wherein the polymer is a biostable polymer.

6. A method according to claim 5 wherein the polymer is selected from the group consisting of silicones, polyurethanes, polyesters, vinyl homopolymers and copolymers, acrylate homopolymers and copolymers, polyethers and cellulosics.

7. A method according to claim 1 wherein the ratio of drug to dissolved polymer in the solution is in the range of about 10:1 to 1:100.

8. A method according to claim 1 wherein the drug is selected from the groups consisting of glucocorticoids, dexamethasone, dexamethasone sodium phosphate, anticoagulants, heparin, hirudin, tick anticoagulant peptide, angiopeptin, antimitotic agents, and oligonucleotides.

9. A method for making an intravascular stent comprising the steps of:

(a) providing a cylindrical, radially expandable stent body;

(b) applying to the stent body a solution which includes a solvent, a bioabsorbable polymer dissolved in the solvent and a therapeutic substance dispersed in the solvent;

(c) evaporating the solvent;

(d) repeating application and evaporating steps (b) and (c) to provide an amount of polymer and therapeutic substance on the stent body; and

(e) radially expanding the stent body and applied polymer and therapeutic substance such that the polymer and therapeutic substance are retained on the stent body.

10. A method according to claim 9 wherein the polymer is selected from the group consisting of poly(L-lactic acid), poly(lactide-co-glycolide) and poly(hydroxybutyrate-co-valerate).

11. A method according to claim 9 wherein the stent body has a metal surface.

12. A method according to claim 9 wherein the stent body has a polymeric surface.

13. A method according to claim 9 wherein the solution is applied by spraying.

14. A method according to claim 9 wherein the solution is applied by immersion.

15. A method according to claim 9 wherein the ratio of drug to dissolved polymer in the solution is varied in some of the plurality of application steps.

16. A method according to claim 9 wherein the ratio of drug to dissolved polymer in the solution is in the range of about 10:1 to 1:100.

17. A method according to claim 9 wherein the drug is selected from the groups consisting of glucocorticoids, dexamethasone, dexamethasone sodium phosphate, anticoagulants, heparin, hirudin, tick anticoagulant peptide, angiopeptin, antimitotic agents, and oligonucleotides.

18. A method for making an intravascular stent comprising the steps of:

(a) providing a cylindrical, radially expandable, metal stent body;

(b) spraying onto the stent body a solution which includes a solvent, a bioabsorbable polymer dissolved in the solvent and a glucocorticoid dispersed in the solvent; and

(c) evaporating the solvent; and

(d) radially expanding the stent body and applied polymer and glucocorticoid such that the polymer and glucocorticoid are retained on the stent body.

19. A method according to claim 18 wherein the solution is applied in a plurality of application and drying steps.

20. A method according to claim 19 wherein the ratio of drug to dissolved polymer in the solution is varied in some of the plurality of application steps.

21. A method according to claim 18 wherein the polymer is selected from the group consisting of poly(L-lactic acid), poly(lactide-co-glycolide), fibrin, silicone, polyurethane, and poly(phosphoester urethane).

22. A method according to claim 18 wherein the glucocorticoid is dexamethasone.

* * * * *

US005508286A

United States Patent [19]

[11] **Patent Number: 5,508,286**

[45] **Date of Patent: Apr. 16, 1996**

Skotnicki et al.

[54] **RAPAMYCIN AMIDINO CARBAMATES**

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[73] Assignee: **American Home Products Corporation**, Madison, N.J.

[21] Appl. No.: **450,771**

[22] Filed: **May 24, 1995**

Related U.S. Application Data

[62] Division of Ser. No. 259,763, Jun. 14, 1994, Pat. No. 5,463,048.

[51] **Int. Cl.**^{6} **A61K 31/395**; C07D 498/16

[52] **U.S. Cl.** **514/291**; 540/456

[58] **Field of Search** 540/456; 514/291

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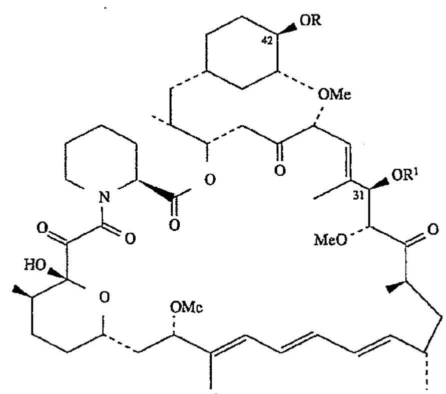
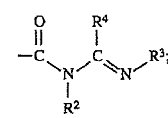
Primary Examiner—Robert T. Bond
Attorney, Agent, or Firm—Arnold S. Milowsky

[57] **ABSTRACT**

A compound of the structure



wherein R and R^1 are each, independently, hydrogen, or



R^2 and R^3 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, $-CO_2R^5$, $-COR^5$, $-CN$, $-NO_2$, $-SO_2R^5$, $-SO_3R^5$, $-OR^5$, $-SR^5$, or Ar;

R^4 is hydrogen, alkyl, alkenyl, alkynyl, $-CF_3$, $-NR^5R^6$, $-CO_2R^5$, $-COR^5$, $CONR^5R^6$, $-NO_2$, halogen, $-OR^5$, $-SR^5$, $-CN$, $-SO_2R^5$, $-SO_3R^5$, $-SO_2NR^5R^6$, or Ar;

R^5 and R^6 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, or Ar;

Ar is phenyl, naphthyl, or hetaryl, wherein the foregoing may be optionally substituted; with the proviso that R and R^1 are both not hydrogen, or a pharmaceutically acceptable salt thereof which is useful as an immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agent.

1 Claim, No Drawings

RAPAMYCIN AMIDINO CARBAMATES

This is a division of application Ser. No. 08/259,763 filed Jun. 14, 1994, now U.S. Pat. No. 5,463,048.

BACKGROUND OF THE INVENTION

This invention relates to amidino carbamates of rapamycin and a method for using them for inducing immunosuppression, and in the treatment of transplantation rejection, graft vs. host disease, autoimmune diseases, diseases of inflammation, adult T-cell leukemia/lymphoma, solid tumors, fungal infections, and hyperproliferative vascular disorders.

Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Pat. No. 3,929,992; and U.S. Pat. No. 3,993,749].

Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

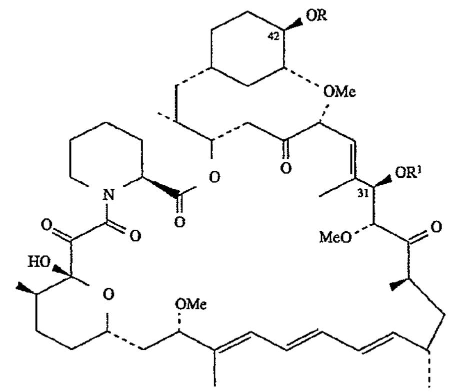
The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Pat. No. 5,100,899].

Rapamycin has also been shown to be useful in preventing or treating systemic lupus erythematosus [U.S. Pat. No. 5,078,999], pulmonary inflammation [U.S. Pat. No. 5,080,899], insulin dependent diabetes mellitus [Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract), (1990)], smooth muscle cell proliferation and intimal thickening following vascular injury [Morris, R. J. Heart Lung Transplant 11 (pt. 2): 197 (1992)], adult T-cell leukemia/lymphoma [European Patent Application 525,960 A1], and ocular inflammation [European Patent Application 532,862 A1].

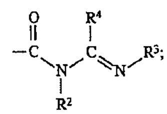
Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble aminoacyl prodrugs of rapamycin (U.S. Pat. No. 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions. U.S. Pat. Nos. 5,118,678 and 5,302,584 discloses carbamates of rapamycin that are useful as immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agents.

DESCRIPTION OF THE INVENTION

This invention provides derivatives of rapamycin which are useful as immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agents having the structure



wherein R and R^1 are each, independently, hydrogen, or



R^2 and R^3 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, —CO_2R^5, —COR^5, —CN, —NO_2, —SO_2R^5, —SO_3R^5, —OR^5, —SR^5, or Ar;

R^4 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, —CF_3, —NR^5R^6, —CO_2R^5, —COR^5, $CONR^5R^6$, —NO_2, halogen, —OR^5, —SR^5, —CN, —SO_2R^5, —SO_3R^5, —$SO_2NR^5R^6$, or Ar;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or Ar;

Ar is phenyl, naphthyl, or hetaryl, wherein the foregoing may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, —SO_3H, and —CO_2H;

with the proviso that R and R^1 are both not hydrogen, or a pharmaceutically acceptable salt thereof.

The pharmaceutically acceptable salts are those derived from such inorganic cations such as sodium, potassium, and the like; organic bases such as: mono-, di-, and trialkyl amines of 1–6 carbon atoms, per alkyl group and mono-, di-, and trihydroxyalkyl amines of 1–6 carbon atoms per alkyl group, and the like; and organic and inorganic acids as: acetic, lactic, citric, tartaric, succinic, maleic, malonic, gluconic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, and similarly known acceptable acids.

The terms alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, and alkynyl of 2–7 carbon atoms, include both straight chain as well as branched carbon chains. When any of the generic terms (i.e., R^5) are contained more than once in a given compound, each may be the same or different.

Hetaryl is defined as an unsaturated or partially saturated heterocyclic radical of 5–12 atoms having 1 ring or 2 fused